Alexa Fluor® 700 anti-human CD3 Antibody

Catalog# / Size	300423 / 25 µg 300424 / 100 µg
Clone	UCHT1
Regulatory Status	RUO
Workshop	III 471
Other Names	T3, CD3ε
Isotype	Mouse IgG1, κ
Description	CD3ε is a 20 kD chain of the CD3/T-cell receptor (TCR) complex which is composed of two CD3ε, one CD3γ, one CD3δ, one CD3ζ (CD247), and a T-cell receptor (α/β or γ/δ) heterodimer. It is found on all mature T cells, NKT cells, and some thymocytes. CD3, also known as T3, is a member of the immunoglobulin superfamily that plays a role in antigen recognition, signal transduction, and T cell activation.

Product Details

Verified Reactivity	Human
Reported Reactivity	Chimpanzee
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 700 under optimal conditions.
Concentration	0.5 mg/ml
Storage & Handling	The CD3 antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	FC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. The suggested use of this reagent is ≤1.0 µg per million cells in 100 µl volume. It is highly recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 700 has a maximum emission of 719 nm when it is excited at 633 nm / 635 nm. Prior to using Alexa Fluor® 700 conjugate for flow cytometric analysis, please verify your flow cytometer's capability of exciting and detecting the fluorochrome. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Red Laser (633 nm)
Application Notes	Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen sections[4,6,7] and formalin-fixed paraffin-embedded sections[11], immunoprecipitation[1], activation of T cells[2,3,5], Western blotting[9], and spatial biology (IBEX)[16,17]. The LEAF™ purified antibody (Endotoxin < 0.1 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for functional assays (Cat. No. 300413, 300414, and 300432). For highly sensitive assays, we recommend Ultra-LEAF™ purified antibody (Cat. No. 300437, 300438, 300465, 300466, 300473, 300474) with a lower endotoxin limit than standard LEAF™ purified antibodies (Endotoxin < 0.01 EU/µg).
Application References	1. Salmeron A, *et al.* 1991. *J. Immunol.* 147:3047. (IP)

(PubMed link indicates BioLegend citation)

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RRID

AB_493740 (BioLegend Cat. No. 300423)
AB_493741 (BioLegend Cat. No. 300424)

Antigen Details

Structure

Ig superfamily, with the subunits of CD3γ, CD3δ, CD3ζ (CD247) and TCR (α/β or γ/δ) forms

	CD3/TCR complex, 20 kD
Distribution	Mature T and NK T cells, thymocyte differentiation
Function	Antigen recognition, signal transduction, T cell activation
Ligand/Receptor	Peptide antigen bound to MHC
Cell Type	NKT cells, T cells, Thymocytes, Tregs
Biology Area	Immunology, Innate Immunity
Molecular Family	CD Molecules, TCRs
Antigen References	1. Barclay N, *et al.* 1993. The Leucocyte FactsBook. Academic Press. San Diego. 2. Beverly P, *et al.* 1981. *Eur. J. Immunol.* 11:329. 3. Lanier L, *et al.* 1986. *J. Immunol.* 137:2501-2507.
Gene ID	916

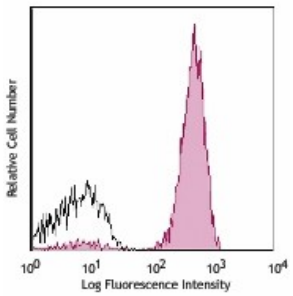
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

Other Formats

APC anti-human CD3, Biotin anti-human CD3, FITC anti-human CD3, PE anti-human CD3, PE/Cyanine5 anti-human CD3, Purified anti-human CD3, Alexa Fluor® 647 anti-human CD3, Alexa Fluor® 488 anti-human CD3, Pacific Blue™ anti-human CD3, PE/Cyanine7 anti-human CD3, Alexa Fluor® 700 anti-human CD3, APC/Cyanine7 anti-human CD3, PerCP anti-human CD3, PerCP/Cyanine5.5 anti-human CD3, Brilliant Violet 421™ anti-human CD3, Brilliant Violet 570™ anti-human CD3, Ultra-LEAF™ Purified anti-human CD3, Purified anti-human CD3 (Maxpar® Ready), Alexa Fluor® 594 anti-human CD3, PE/Dazzle™ 594 anti-human CD3, Brilliant Violet 510™ anti-human CD3, Brilliant Violet 605™ anti-human CD3, Brilliant Violet 711™ anti-human CD3, Brilliant Violet 650™ anti-human CD3, APC/Fire™ 750 anti-human CD3, Brilliant Violet 785™ anti-human CD3, TotalSeq™-A0034 anti-human CD3, TotalSeq™-B0034 anti-human CD3, TotalSeq™-C0034 anti-human CD3, KIRAVIA Blue 520™ anti-human CD3, Spark Violet™ 538 anti-human CD3 Antibody, TotalSeq™-D0034 anti-human CD3, Spark Blue™ 574 anti-human CD3 Antibody, GMP Pacific Blue™ anti-human CD3, GMP PE anti-human CD3, GMP PE/Dazzle™ 594 anti-human CD3

Product Data



Human peripheral blood lymphocytes stained with UCHT1 Alexa Fluor® 700

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CD8 (SK1)

FORMS

Form	Catalog number	Form	Catalog number	Form	Catalog number
Pure	340855	PE-Cy7	335805	AmCyan	339199
FITC	340692	APC	340659	V500-C	647458
PerCP	340693	APC-Cy7	348803	BV605	663798
PerCP-Cy5.5	341049	APC-H7	641409	BV786	664530

DESCRIPTION

Specificity

The CD8 antibody recognizes the 32-kilodalton (kDa) α-subunit of a disulfide-linked bimolecular complex.[1,2] The majority of peripheral blood $CD8^+$ T lymphocytes expresses an α/β heterodimer (32, 30 kDa), while $CD8^+CD16^+$ natural killer (NK) lymphocytes and $CD8^+$ T-cell receptor (TCR)-γ/δ^+ T lymphocytes express an α/α homodimer (30 kDa). $CD8^+$TCR-α/β^+ T lymphocytes can express either an α/α homodimer or α/β heterodimer.[1,2] The CD8 antigenic determinant binds to class I major histocompatibility (MHC) molecules, resulting in increased adhesion between the $CD8^+$ T lymphocytes and target cells.[3-5] Binding of the CD8 antigen to class I MHC molecules enhances the activation of resting T lymphocytes.[3-6] The CD8 antigen is coupled to a protein tyrosine kinase, $p56^{lck}$. The CD8:$p56^{lck}$ complex can play a role in T-lymphocyte activation through mediation of the interactions between the CD8 antigen and the CD3/TCR complex.[5,6].

Antigen distribution

The CD8 antigen is present on the human suppressor/cytotoxic T-lymphocyte subset[7-12] as well as on a subset of NK lymphocytes.[13] The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes[14] and 60% to 85% of normal thymocytes.[7,11] The $CD8^+$ T- and NK-lymphocyte subsets can be further subdivided into the following groups: $CD16^+$ NK lymphocytes that can express the CD8 antigen in low density;[13] $CD57^+$ T lymphocytes that express high-density CD8 antigen;[13] and $CD8^+CD62L^+$ lymphocytes that collaborate with $CD8^+CD62L^-$ lymphocytes to generate suppression of B-lymphocyte function.[15] CD8 cross-reacts with lymphocytes of some nonhuman primate species.[16].

Clone

The CD8 antibody, clone SK1[17], is derived from the hybridization of NS-1 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with human peripheral blood T lymphocytes.

Composition

The CD8 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Analyte Specific Reagent. Analytical and performance characteristics are not established.

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1/2021 23-3729-11

Product configuration

The following are supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
Pure	200	20	50	4.0	12.5	Gelatin	0.1% Sodium azide
FITC	100	20	25	2.0	12.5	Gelatin	0.1% Sodium azide
PerCP	100	20	12	2.0	6	Gelatin	0.1% Sodium azide
PerCP-Cy5.5	50	20	5	1.0	5	Gelatin	0.1% Sodium azide
PE-Cy7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
APC	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
APC-Cy7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
APC-H7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
AmCyan	100	5	50	0.5	100	BSA	0.1% Sodium azide
V500-C[a]	100	5	25	0.5	50	BSA	ProClin™ 950
BV605[a]	100	5	25	0.5	50	BSA	0.09% Sodium azide
BV786[a]	100	5	25	0.5	50	BSA	0.09% Sodium azide

a. BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 605, BD Horizon Brilliant™ Violet 711

CAUTION Some APC-Cy7 conjugates, and to a lesser extent PE-Cy7 and APC-H7 conjugates, show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

CAUTION If you choose to combine BD Horizon Brilliant™ reagents in a multicolor staining cocktail, dyes might bind to one another without the use of a buffering solution, such as BD Horizon™ Brilliant Stain Buffer.

Purity

Pure: ≥85% pure at bottling, as measured by polyacrylamide gel electrophoresis (PAGE)

FITC: ≤5% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)

PerCP, PerCP-Cy5.5, PE-Cy7, APC, APC-Cy7, APC-H7, AmCyan, V500-C: ≤20% free fluorophore at bottling, as measured by SEC

BV605, BV786: ≤25% free fluorophore at bottling, as measured by ion-exchange chromatography (IEC)

HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[18,19] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Go to regdocs.bd.com to download the Safety Data Sheet.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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PATENTS AND TRADEMARKS

BV605 and BV786 covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,575,303; or 8,354,239.

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Brilliant Violet 421™ anti-human CD8 Antibody

Catalog# / Size	344747 / 25 tests 344748 / 100 tests
Clone	SK1
Regulatory Status	RUO
Other Names	T8, Leu2
Isotype	Mouse IgG1, κ
Description	CD8a is a 32-34 kD type I glycoprotein. It forms a homodimer (CD8a/a) or heterodimer (CD8a/b) with CD8b. CD8, also known as T8 and Leu2, is a member of the immunoglobulin superfamily found on the majority of thymocytes, a subset of peripheral blood T cells, and NK cells (which express almost exclusively CD8a homodimers). CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition and T cell activation and has been shown to play a role in thymic differentiation. Two domains in CD8a are important for function: the extracellular IgSF domain binds the α_3 domain of MHC class I and the cytoplasmic CXCP motif binds the tyrosine kinase p56 Lck.

Product Details

Verified Reactivity	Human, Cynomolgus, Rhesus
Reported Reactivity	African Green, Chimpanzee, Pigtailed Macaque, Sooty Mangabey
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA).
Preparation	The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 421™ under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	FC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. Brilliant Violet 421™ excites at 405 nm and emits at 421 nm. The standard bandpass filter 450/50 nm is recommended for detection. Brilliant Violet 421™ is a trademark of Sirigen Group Ltd. Learn more about Brilliant Violet™. This product is subject to proprietary rights of Sirigen Inc. and is made and sold under license from Sirigen Inc. The purchase of this product conveys to the buyer a non-transferable right to use the purchased product for research purposes only. This product may not be resold or incorporated in any manner into another product for resale. Any use for therapeutics or diagnostics is strictly prohibited. This product is covered by U.S. Patent(s), pending patent applications and foreign equivalents.
Excitation Laser	Violet Laser (405 nm)
Application Notes	Clone SK1 recognizes the a chain of CD8. Additional reported applications (for the relevant formats) include: proteogenomics[8], immunohistochemistry of acetone-fixed frozen tissue sections, and spatial biology (IBEX)[9,10]. This clone was tested in-house and does not demonstrate utility for formalin-fixed paraffin-embedded (FFPE) human tonsil sections.

Application References

(PubMed link indicates BioLegend citation)

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RRID

AB_2629583 (BioLegend Cat. No. 344747)
AB_2629584 (BioLegend Cat. No. 344748)

Antigen Details

Structure	Ig superfamily, homodimer or heterodimer with CD8b, 32-34 kD
Distribution	Majority of thymocytes, T cell subset, NK cells
Function	MHC class I co-receptor, thymic differentiation, T cell activation
Ligand/Receptor	MHC Class I molecules
Cell Type	NK cells, T cells, Thymocytes
Biology Area	Immunology
Molecular Family	CD Molecules
Antigen References	1. Barclay N, *et al.* 1993. *The Leucocyte Antigen FactsBook*. Academic Press Inc. San Diego.
Gene ID	925

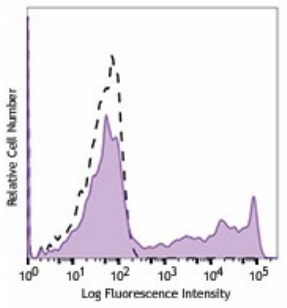
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

Other Formats

Alexa Fluor® 647 anti-human CD8, Brilliant Violet 650™ anti-human CD8, Purified anti-human CD8, FITC anti-human CD8, PE anti-human CD8, PerCP anti-human CD8, PerCP/Cyanine5.5 anti-human CD8, PE/Cyanine7 anti-human CD8, APC/Cyanine7 anti-human CD8, Alexa Fluor® 488 anti-human CD8, Pacific Blue™ anti-human CD8, Biotin anti-human CD8, APC anti-human CD8, Alexa Fluor® 700 anti-human CD8, Purified anti-human CD8 (Maxpar® Ready), Brilliant Violet 510™ anti-human CD8, Brilliant Violet 711™ anti-human CD8, Brilliant Violet 785™ anti-human CD8, Brilliant Violet 605™ anti-human CD8, PE/Dazzle™ 594 anti-human CD8, APC/Fire™ 750 anti-human CD8, Brilliant Violet 421™ anti-human CD8, TotalSeq™-A0046 anti-human CD8, TotalSeq™-C0046 anti-human CD8, Brilliant Violet 750™ anti-human CD8, TotalSeq™-B0046 anti-human CD8, Spark Blue™ 550 anti-human CD8, APC/Fire™ 810 anti-human CD8, PE/Fire™ 640 anti-human CD8, PE/Fire™ 700 anti-human CD8, TotalSeq™-D0046 anti-human CD8, GMP APC anti-human CD8, PE/Cyanine5 anti-human CD8 Antibody, Spark UV™ 387 anti-human CD8, GMP PE anti-human CD8, GMP PE/Cyanine7 anti-human CD8, Spark NIR™ 685 anti-human CD8, KIRAVIA Blue 520™ anti-human CD8, GMP FITC anti-human CD8, GMP Pacific Blue™ anti-human CD8, GMP PerCP anti-human CD8, Spark Violet™ 500 anti-human CD8

Product Data



Human peripheral blood lymphocytes were stained with CD8 (clone SK1) Brilliant Violet 421™ (filled histogram) or mouse IgG1, κ Brilliant Violet 421™ isotype control (open histogram).

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PE/Dazzle™ 594 anti-human CD197 (CCR7) Antibody

Catalog# / Size	353235 / 25 tests 353236 / 100 tests
Clone	G043H7
Regulatory Status	RUO
Other Names	BLR2, CDw197, EBI1, CMKBR7
Isotype	Mouse IgG2a, κ
Description	CCR7, also known as CD197, is a chemokine receptor that binds CCL19 and CCL21. CCR7 and its ligands link innate and adaptive immunity by affecting interactions between T cells and dendritic cells and their downstream effect. Naïve T cells enter the lymph node through high endothelial venules, which express CCL21. Dendritic cells and macrophages enter the lymph node through afferent lymphatics. The encounter of T cells and dendritic cells in the T cell zone is CCR7-dependent. In addition, during immunological surveillance, B cells recirculate between B-cell-rich compartments (follicles or B cell zones) in secondary lymphoid organs, surveying for antigen. After antigen binding, B cells move to the boundary of B and T zones to interact with T-helper cells; this B cell migration is directed by CCR7 and its ligands. CCR7-positive cancer cell expression has been associated with lymph node metastasis.

Product Details

Verified Reactivity	Human
Reported Reactivity	African Green, Baboon, Cynomolgus, Rhesus
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	CCR7-transfected cells
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with PE/Dazzle™ 594 under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	FC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. * PE/Dazzle™ 594 has a maximum excitation of 566 nm and a maximum emission of 610 nm.
Excitation Laser	Blue Laser (488 nm) Green Laser (532 nm)/Yellow-Green Laser (561 nm)

Product Citations

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2. Jiguet-Jiglaire C, *et al.* 2022. Acta Neuropathol Commun. 10:1. PubMed
3. Mogilenko DA, *et al.* 2020. Immunity. 54(1):99-115.e12. PubMed
4. Neidleman J, *et al.* 2020. Elife. 9:00. PubMed
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10. Menges D, *et al.* 2022. Nat Commun. 13:4855. PubMed
11. FitzPatrick MEB, *et al.* 2021. Cell Rep. 34:108661. PubMed
12. Duhen R, *et al.* 2021. Nat Commun. 12:1047. PubMed
13. Xie G, *et al.* 2021. Cell Reports. 35(4):109038. PubMed
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RRID

AB_2563640 (BioLegend Cat. No. 353235)
AB_2563641 (BioLegend Cat. No. 353236)

Antigen Details

Structure

Chemokine receptor, G protein-coupled receptors (GPCR), seven transmembrane receptor.

Distribution

T cells, B cells, NK, dendritic cells.

Function

The chemokine receptor CCR7 plays a pivotal role in the homing of naïve T cells and regulatory T cells to secondary lymphoid organs, and the migration of dendritic cells into afferent lymphatic vessels.

Ligand/Receptor

CCL19 and CCL21.

Cell Type

B cells, Dendritic cells, NK cells, T cells

Biology Area

Immunology

Molecular Family

CD Molecules, Cytokine/Chemokine Receptors, GPCR

Antigen References

1. Yanagihara S, *et al.* 1998. *J. Immunol.* 161:3096.
2. Charo IF, *et al.* 2006. *N. Engl. J. Med.* 354:610.
3. Reif K, *et al.* 2002. *Nature* 416:94.
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5. Brodie T. *et al.* 2013. *Cytometry A.* 6: 530-2. PubMed
6. Graves A.J. et al. 2014. Cytometry A. 7: 576–9 PubMed
7. Moncunill G. et al. 2014. Cytometry A. 12: 995-8 PubMed

Gene ID

1236

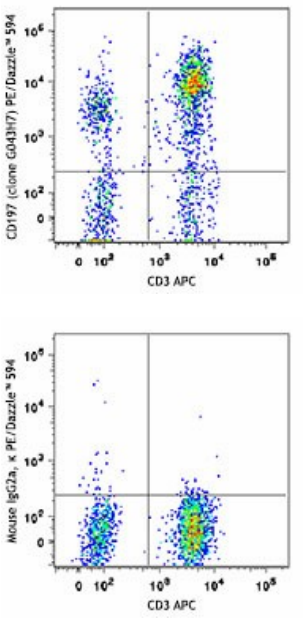
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

Other Formats

Purified anti-human CD197 (CCR7), Alexa Fluor® 488 anti-human CD197 (CCR7), Brilliant Violet 421™ anti-human CD197 (CCR7), PE anti-human CD197 (CCR7), APC/Cyanine7 anti-human CD197 (CCR7), Pacific Blue™ anti-human CD197 (CCR7), APC anti-human CD197 (CCR7), FITC anti-human CD197 (CCR7), Alexa Fluor® 647 anti-human CD197 (CCR7), PerCP/Cyanine5.5 anti-human CD197 (CCR7), Brilliant Violet 605™ anti-human CD197 (CCR7), PE/Cyanine7 anti-human CD197 (CCR7), Brilliant Violet 711™ anti-human CD197 (CCR7), Brilliant Violet 785™ anti-human CD197 (CCR7), Brilliant Violet 510™ anti-human CD197 (CCR7), Brilliant Violet 650™ anti-human CD197 (CCR7), PE/Dazzle™ 594 anti-human CD197 (CCR7), Biotin anti-human CD197 (CCR7), Purified anti-human CD197 (CCR7) (Maxpar® Ready), PerCP anti-human CD197 (CCR7), Alexa Fluor® 700 anti-human CD197 (CCR7), APC/Fire™ 750 anti-human CD197 (CCR7), TotalSeq™-A0148 anti-human CD197 (CCR7), TotalSeq™-B0148 anti-human CD197 (CCR7), TotalSeq™-C0148 anti-human CD197 (CCR7), Brilliant Violet 750™ anti-human CD197 (CCR7), Ultra-LEAF™ Purified anti-human CD197 (CCR7), Spark NIR™ 685 anti-human CD197 (CCR7), KIRAVIA Blue 520™ anti-human CD197 (CCR7), PE/Fire™ 640 anti-human CD197 (CCR7), Spark YG™ 581 anti-human CD197 (CCR7), APC/Fire™ 810 anti-human CD197 (CCR7) Antibody, TotalSeq™-D0148 anti-human CD197 (CCR7), PE/Fire™ 810 anti-human CD197 (CCR7) Antibody, PE/Cyanine5 anti-human CD197 (CCR7)

Product Data



Human peripheral blood lymphocytes were stained with CD3 APC and CD197 (clone G043H7) PE/Dazzle™ 594 (top) or mouse IgG2a, κ PE/Dazzle™ 594 isotype control (bottom).

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PE anti-human CD161 Antibody

Catalog# / Size	339903 / 25 tests 339904 / 100 tests 339938 / 100 µg
Clone	HP-3G10
Regulatory Status	RUO
Other Names	NKR-P1A
Isotype	Mouse IgG1, κ
Description	CD161 is a type II transmembrane glycoprotein, also known as NKR-P1A, that is expressed as a 40-44 kD homodimer. It is a member of the C-type lectin superfamily. CD161 is expressed on a majority of NK cells, NKT cells, and subsets of peripheral T cells and $CD3^+$ thymocytes. It has been reported that Th17 cells are a subpopulation of $CD4^+CD161^+CCR6^+$ cells. While the biological function of CD161 is not clear, it has been suggested to serve either as a stimulatory receptor or to inhibit NK cell-mediated cytotoxicity and cytokine production. LLT-1 (lectin-like transcript-1, also named as osteoclast inhibitory lectin or CLEC2D) is the ligand of CD161.

Product Details

Verified Reactivity	Human, Cynomolgus, Rhesus
Reported Reactivity	African Green, Baboon
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	Human NK cells
Formulation	µg size: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide. test sizes: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA).
Preparation	The antibody was purified by affinity chromatography, and conjugated with PE under optimal conditions.
Concentration	µg sizes: 0.2 mg/mL test sizes: lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	FC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining using the µg size, the suggested use of this reagent is ≤1.0 µg per million cells in 100 µl volume. It is recommended that the reagent be titrated for optimal performance for each application. For flow cytometric staining using the test sizes, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.
Excitation Laser	Blue Laser (488 nm) Green Laser (532 nm)/Yellow-Green Laser (561 nm)
Application Notes	Additional reported applications (for the relevant formats) include: inhibition of cytokine production and Western blotting under nonreducing conditions.
Application References **(PubMed link indicates BioLegend citation)**	1. Gumß M, et al. 2004. *Blood* 104:3664. 2. Exley M, *et al.* 1998. *J. Exp. Med.* 188:867. 3. Marquez C, *et al.* 1998. *Blood* 91:2760.

Product Citations

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RRID

AB_1501086 (BioLegend Cat. No. 339903)
AB_1501083 (BioLegend Cat. No. 339904)
AB_2564141 (BioLegend Cat. No. 339938)

Antigen Details

Structure	Type II glycoprotein, 40-44 kD homodimer, C-type lectin superfamily
Distribution	NK cells, T subset, subset of $CD3^+$ thymocytes
Ligand/Receptor	LLT-1 (Lectin-like transcript-1)
Cell Type	NK cells, T cells, Thymocytes
Biology Area	Cell Biology, Immunology, Innate Immunity, Signal Transduction
Molecular Family	CD Molecules
Antigen References	1. Takahashi T, *et al.* 2006. *J. Immunol.* 176:211. 2. Cosmi L, *et al.* 2008. *J. Exp. Med.* 205:1903. 3. Aldemir H, *et al.* 2005. *J. Immunol.* 175:7791. 4. Rosen DB, *et al.* 2008. *J. Immunol.* 180:6508.
Gene ID	3820

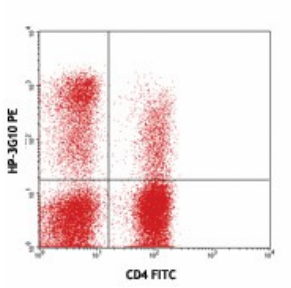
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

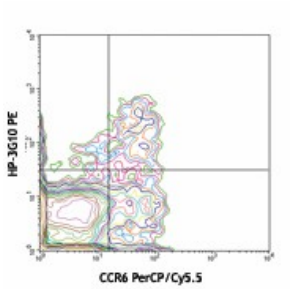
Other Formats

Brilliant Violet 510™ anti-human CD161, FITC anti-human CD161, Purified anti-human CD161, PE anti-human CD161, PerCP/Cyanine5.5 anti-human CD161, Alexa Fluor® 647 anti-human CD161, APC anti-human CD161, Brilliant Violet 421™ anti-human CD161, Brilliant Violet 605™ anti-human CD161, PE/Cyanine7 anti-human CD161, Alexa Fluor® 700 anti-human CD161, Purified anti-human CD161 (Maxpar® Ready), Alexa Fluor® 488 anti-human CD161, Pacific Blue™ anti-human CD161, APC/Cyanine7 anti-human CD161, Brilliant Violet 785™ anti-human CD161, Biotin anti-human CD161, PerCP anti-human CD161, PE/Dazzle™ 594 anti-human CD161, APC/Fire™ 750 anti-human CD161, TotalSeq™-A0149 anti-human CD161, TotalSeq™-C0149 anti-human CD161, TotalSeq™-B0149 anti-human CD161, PE/Cyanine5 anti-human CD161 Antibody, TotalSeq™-D0149 anti-human CD161, Spark Red™ 718 anti-human CD161

Product Data



Human peripheral blood lymphocytes stained with CD4 FITC and HP-3G10 PE



Human peripheral blood CD4+ lymphocytes stained with CCR6 PerCP/Cy5.5 and HP-3G10 PE

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PE/Cyanine7 anti-human CD45RA Antibody

Catalog# / Size	304125 / 25 tests 304126 / 100 tests
Clone	HI100
Regulatory Status	RUO
Workshop	IV N906
Other Names	GP180, L-CA, LCA, LY5, T200, PTPRC
Isotype	Mouse IgG2b, κ
Description	CD45RA is a 205-220 kD single chain type I glycoprotein. It is an exon 4 splice variant of the tyrosine phosphatase CD45. The CD45RA isoform is expressed on resting/naïve T cells, medullary thymocytes, B cells and monocytes. CD45RA enhances both T cell receptor and B cell receptor signaling. CD45 non-covalently associates with lymphocyte phosphatase-associated phosphoprotein (LPAP) on T and B lymphocytes. CD45 has been reported to be associated with several other cell surface antigens including CD1, CD2, CD3, and CD4. CD45 has also been reported to bind galectin-1. CD45 isoform expression can change in response to cytokines.

Product Details

Verified Reactivity	Human
Reported Reactivity	Chimpanzee
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with PE/Cyanine7 under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	FC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.
Excitation Laser	Blue Laser (488 nm) Green Laser (532 nm)/Yellow-Green Laser (561 nm)
Application Notes	Additional reported applications (for relevant formats of this clone) include: inhibition of CD45 functions[2], immunohistochemical staining of frozen tissue sections[3] and formalin-fixed paraffin-embedded tissue sections[4], and immunocytochemistry[15,16].
Additional Product Notes	BioLegend is in the process of converting the name PE/Cy7 to PE/Cyanine7. The dye molecule remains the same, so you should expect the same quality and performance from our PE/Cyanine7 products. Please contact Technical Service if you have any questions.

Application References

(PubMed link indicates BioLegend citation)

1. Knapp W, *et al.* 1989. Leucocyte Typing IV. Oxford University Press. New York.
2. Yamada T, *et al.* 2002. *J. Biol. Chem.* 277:28830. (WB, Block)
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Product Citations

1. Jiang XL, *et al.* 2021. Nat Commun. 12:897. PubMed
2. Robinson GA, *et al.* 2021. EBioMedicine. 103243:. PubMed
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RRID

AB_10709440 (BioLegend Cat. No. 304125)
AB_10708879 (BioLegend Cat. No. 304126)

Antigen Details

Structure	Tyrosine phosphatases, type I transmembrane (exon 4 splicing of CD45 gene), 205-220 kD
Distribution	B cells, naïve T cells, monocytes
Function	Enhances TCR and BCR signaling
Ligand/Receptor	Galectin-1, CD2, CD3, CD4
Cell Type	B cells, Monocytes, T cells, Tregs
Biology Area	Cell Biology, Immunology, Inhibitory Molecules, Neuroscience, Neuroscience Cell Markers
Molecular Family	CD Molecules
Antigen References	1. Thomas M. 1989. *Annu. Rev. Immunol.* 7:339. 2. Trowbridge I, *et al.* 1994. *Annu. Rev. Immunol.*12:85.

Gene ID 5788

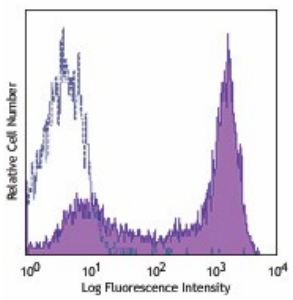
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

Other Formats

APC anti-human CD45RA, Biotin anti-human CD45RA, FITC anti-human CD45RA, PE anti-human CD45RA, PE/Cyanine5 anti-human CD45RA, Purified anti-human CD45RA, Alexa Fluor® 488 anti-human CD45RA, Alexa Fluor® 647 anti-human CD45RA, Pacific Blue™ anti-human CD45RA, Alexa Fluor® 700 anti-human CD45RA, PerCP/Cyanine5.5 anti-human CD45RA, PE/Cyanine7 anti-human CD45RA, APC/Cyanine7 anti-human CD45RA, Brilliant Violet 421™ anti-human CD45RA, Brilliant Violet 570™ anti-human CD45RA, Brilliant Violet 605™ anti-human CD45RA, Brilliant Violet 650™ anti-human CD45RA, Brilliant Violet 711™ anti-human CD45RA, Brilliant Violet 785™ anti-human CD45RA, Brilliant Violet 510™ anti-human CD45RA, Purified anti-human CD45RA (Maxpar® Ready), PE/Dazzle™ 594 anti-human CD45RA, APC/Fire™ 750 anti-human CD45RA, PerCP anti-human CD45RA, TotalSeq™-A0063 anti-human CD45RA, Alexa Fluor® 594 anti-human CD45RA, TotalSeq™-B0063 anti-human CD45RA, TotalSeq™-C0063 anti-human CD45RA, Brilliant Violet 750™ anti-human CD45RA, Spark NIR™ 685 anti-human CD45RA, PE/Fire™ 640 anti-human CD45RA, PE/Fire™ 700 anti-human CD45RA Antibody, Spark YG™ 581 anti-human CD45RA, TotalSeq™-D0063 anti-human CD45RA, Spark Violet™ 423 anti-human CD45RA, GMP FITC anti-human CD45RA, Spark UV™ 387 anti-human CD45RA

Product Data



Human peripheral blood lymphocytes were stained with HI100 PE/Cyanine7 (filled histogram) or mouse IgG2b, κ PE/Cyanine7 isotype control (open histogram).

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Monoclonal Antibodies Detecting Human Antigens

CD45RA (L48)

Form	Catalog number
FITC	347723
PE-Cy7	337167

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include studies of:

- Memory T-cell subsets in peripheral blood[1-3]
- T-lymphocyte function[4]
- Immunodeficiency disease[5]
- Multiple sclerosis and other autoimmune diseases[6-8]

DESCRIPTION

Specificity

The CD45RA antibody recognizes a 220-kilodalton (kDa) molecular weight isoform of the leucocyte common antigen (LCA).[9] The CD45RA antigen is a member of the CD45 antigen family that also includes the CD45, CD45RB, and CD45RO antigens.[9]

Antigen distribution

The CD45RA antigen is present on approximately 50% of $CD4^+$ T lymphocytes, approximately 75% of $CD8^+$ T lymphocytes, and on essentially all B lymphocytes and natural killer (NK) lymphocytes.[10] The suppressor inducer T-lymphocyte subset expresses the phenotype $CD4^+CD45RA^+$.[10] The CD45RA antigen is expressed on naive T lymphocytes; antigen density decreases upon in vitro activation.[11] A selective loss of the $CD4^+CD45RA^+$ subset during active multiple sclerosis has been demonstrated.[6,7]

Clone

The CD45RA antibody, clone L48, is derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells from BALB/c mice immunized with low-buoyant–density human lymphocytes.

Composition

The CD45RA antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	100	20	100	2.0	50	Gelatin	0.1% Sodium azide
PE-Cy™7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide

a. Volume required to stain 10^6 cells.

CAUTION Some PE-Cy7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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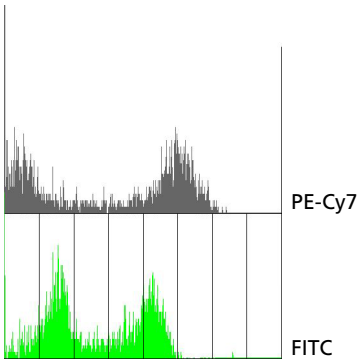
12/2014 23-1439-06

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash method for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on peripheral blood and gated on lymphocytes. Laser excitation was at 488 nm. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following figure.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

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PATENTS AND TRADEMARKS

CD45 检测试剂说明书

【产品名称】

通用名称：CD45 检测试剂

英文名称：CD45- Krome Orange

【包装规格】

100 测试 / 瓶

【预期用途】

检测人体生物标本中 CD45 的表达，为医师提供诊断的辅助信息。

【检验原理】

此实验基于特异性的单克隆抗体与白细胞表面的抗原决定簇特异性结合的原理。白细胞与 CD45- Krome Orange 荧光单克隆抗体试剂孵育反应后，白细胞被特异性染色。然后，通过裂解作用将红细胞去除，白细胞不会受裂解影响而溶解。

流式细胞仪检测细胞的散射光和荧光特性。通过侧向角散射光（SS）和前向角散射光（FS）的关系，在散点图上可以对感兴趣的细胞群设门。流式细胞仪检测的其他不同参数，通过双参数图，也能根据用户的应用，被用于设门。

【主要组成成份】

特异性	CD45
克隆	J33
杂交瘤	NS1 x balb/c
免疫原	Laz 221 细胞系
免疫球蛋白亚型	IgG1 kappa
种属	小鼠
纯化方法	亲和层析
荧光素	Krome Orange
摩尔比	Krome Orange/ Ig: 10.40-14.10
λ 激发	405 nm
发射峰	528 nm
缓冲液	PBS pH 7.2＋2 mg / mL BSA ＋0.1% NaN_3

【储存条件及有效期】

试剂应在 2-8℃避光保存，未开瓶试剂有效期为 24 个月。

以瓶标签失效日期为准，开瓶后可稳定 180 天。

生产日期及失效期见标签。

【适用仪器】

该试剂适用于下列型号的流式细胞仪：FC500、FC500MPL、Navios、DxFlex。

【样本要求】

静脉血要放在经 EDTA 盐处理后无菌试管中，样本放置在 18-25℃，不要摇荡。测试样本前轻柔晃动样本使样本匀质化，样本取到后 24 小时内必须被检测。

【检验方法】

本试剂盒不提供的必需的实验材料：

- 采集样品所需的试管及其它材料
- 移液器和一次性 10，100 和 500µl 吸头
- 塑料溶血管
- 校准微珠：流式细胞仪质控微球（Ref. A63492）流式细胞仪精密度质控微球（Ref. A63493）
- 溶血剂，如：VersaLyse (Ref.A09777)
- 淋巴细胞固定试剂：如：IOTest 3 Fixative solution (Ref. A07800)
- 缓冲液：（PBS: 0.01 M 磷酸钠；0.145 M 氯化钠；pH 7.2）
- 离心机
- 自动混匀器（Vortex 型）
- 流式细胞仪

VERSALYSE 试剂操作流程：

注意：以下程序在标准应用情况下适用。某些 Beckman Coulter 应用时的样品 / 或 VersaLyse 体积可能有所不同。如出现此类情况，按照使用技术说明书中的指导进行操作。

1. 将 10µL 特异性 IOTest 结合抗体加入各个测试试管，并将 10 µL 同种型对照抗体加入各个对照试管。
2. 将 100 µL 测试样品分别加入两组试管。轻微振荡试管。
3. 室温下（18 – 25℃）避光孵育 15 至 20 分钟。
4. 然后裂解红细胞，需按照使用的裂解试剂所附所明书中的建议进行。例如，假如您希望使用 VersaLyse（Ref.A09777），请参阅说明书，最好按照名为“同时固定”的程序实施：包括加入 1 mL 临时配制的“固定-裂解”混合液。立即振荡 1 秒钟，室温下避光孵育 10 分钟。如样品并不含红细胞，则直接加入 2 mL PBS。
5. 室温下 150 x g 离心 5 分钟。
6. 吸去上清液。
7. 将细胞沉淀重悬于 3 mL PBS 中。
8. 重复步骤 5。
9. 吸去上清液，将所获细胞沉淀重悬：

– 如制备液储存时间在 24 小时之内，则使用 0.5 mL 或 1mLPBS ＋0.1%甲醛进行重悬。（0.1%甲醛 PBS 的制备：将 10 倍浓度的 12.5 µL IOTest 3 固定液稀释（Ref.A07800）于 1 mL PBS 中）。

– 如制备液将在 2 小时内进行分析，则使用 0.5 mL PBS（不加入甲醛）进行重悬。

吸去上清液，使用 0.5 mL PBS 将所获细胞沉淀重悬。应在 2 小时内对制剂进行分析。

注意：任何情况下，制备试剂均需在 2 至 8℃下避光保存

【检验结果的解释】

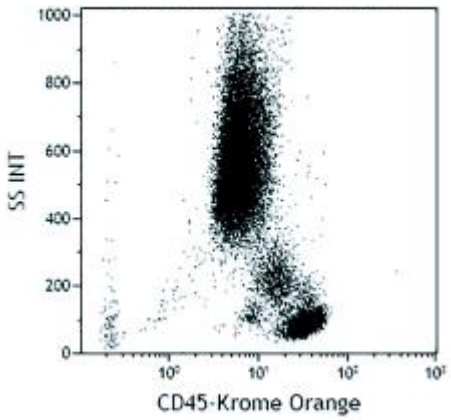
CD45 分子及其亚型在淋巴系（1、2、3）和髓系细胞（4）上有不同程度的表达，这与细胞不同的分化阶段相关，因此 CD45 表达特点可用来区分正常与恶性白细胞（2、4、5、6、7）。在急性髓性白血病时，CD45 表达减少，由此可区分恶性肿瘤细胞与正常细胞（8）。此外，在浆细胞性白血病时，CD45 的表达有评估疾病预后的价值（2、9）。最后，可通过流式散点图上侧向角散射光和 CD45 荧光抗体信号来评价淋巴细胞中有无非白细胞混杂（10）。

示例：

标本用 IOTest CD45-KO 结合抗体染色。

采用配有 Navios 分析软件的 Beckman Coulter Navios 流式细胞仪进行采集的结果。

[白细胞] CD45-Krome Orange/ SS INT



【检验方法的局限性】

1. 如流式细胞仪未正确校正、荧光渗漏未得到正确补偿、以及检测区域未仔细定位，则流式细胞仪可能产生错误的结果。
2. 由于该试剂未针对“无冲洗步骤”裂解技术进行优化，因此，应优先使用包含冲洗步骤的 RBC 裂解技术。
3. 只要操作程序与技术说明书一致，同时符合良好实验室规范要求，所获结果可保持精确度与可重复性。
4. 对该试剂的结合抗体进行了校正，以获得最佳的特异性信号 / 非特异性信号比率。因此，试验中保持要求的试剂体积 / 样品比率是重要的。
5. 如白细胞过多，应使用 PBS 将血样稀释至大约 5 x 10^9 白细胞 / L。（13）
6. 在某些疾病情况下，例如重度肾功能衰竭，或血红蛋白病，红细胞裂解进程可能出现缓慢，裂解不完全，甚至裂解无法进行的情况。一旦出现以上情况，建议染色前使用密度梯度（如 Ficoll）离心法对单核细胞进行分离。（14）
7. CD45 呈阴性或微弱阳性的急性淋巴细胞白血病已有描述。在此情况下，应使用其它标志物对幼稚细胞的淋巴细胞来源进行确认。（15,9）

检测局限性

根据 CLSI EP17-A2《临床实验室测量程序检测能力评价》以及获批指南（第 2 版）（12）进行研究。检出限（LOD）为可持续检出分析物的最低浓度。将获得的结果总结到下表中：

阳性靶标	检出限
淋巴细胞+ CD45-KO	2.0 个细胞 / µL
单核细胞+ CD45-KO	2.0 个细胞 / µL
粒细胞+ CD45-KO	3.0 个细胞 / µL

【产品性能指标】

产品性能数据来自置入 EDTA 盐抗凝剂无菌试管 24 小时并储存在 18-25℃下的血样（使用上述步骤进行处理）。分析于免疫染色后 24 小时内实施。

性能数据来自阳性细胞系。分析于免疫染色后 24 小时内实施。

特异性

CD45 分子为单链内在膜蛋白，至少有 5 种亚型，范围为 180-220kDa。它们是由基因组序列的 3 个外显子（A、B 和 C）选择性剪接组合产生的。非限制性 CD45 抗原，白细胞共同抗原（LCA）包括细胞外序列，位于细胞膜近端，这对所有 CD45 亚型都很常见。所有属于 CD45 簇的单克隆抗体均与抗原该部分反应，能够识别所有 CD45 亚型。这些亚型具有胞浆外序列，序列长度为 391 至 552 个氨基酸残基，具有大量的 N-连接碳水化合物附着位点。胞浆部分包含 2 个磷酸酪氨酸-磷酸酶域。非限制性 CD45 抗原表位存在于所有人白细胞、淋巴细胞、嗜酸性粒细胞单核细胞、嗜碱性粒细胞和中性粒细胞的表面，表达水平依次降低。CD45 是淋巴细胞细胞膜的主要成分。红细胞和血小板中不含 CD45。它在骨髓红系细胞成熟过程中消失。CD45 抗体与骨髓中的白细胞祖细胞反应。J33 单克隆抗体可与人白细胞上存在的所有 CD45 亚型结合。

J33 单克隆抗体在第 3 届国际人体白细胞分化抗原研讨会上（英国牛津，1986 年）被指定为 CD45 分化簇（11）。J33 在 HLDA 3 上被指定为 CD45 簇，命名为 I.33（抗体#818）。

精密度

使用两份全血样本测定阳性靶标的染色百分比，使用相同的细胞仪在同一天重复测定十次。

将获得的结果总结到下表中：

阳性靶标	数量	平均值（%）		SD		CV (%)	
		样本 1	样本 2	样本 1	样本 2	样本 1	样本 2
淋巴细胞+ CD45-KO	10	99.26	99.48	0.20	0.13	0.21	0.13
单核细胞+ CD45-KO	10	99.78	99.92	0.19	0.06	0.19	0.06
粒细胞+ CD45-KO	10	100.00	100.00	0.00	0.00	0.00	0.00

【警告和注意事项】

1. 不要使用过期试剂。
2. 不要冷冻保存试剂。
3. 使用前恢复至室温（18~25℃）。
4. 尽量避光。
5. 避免试剂污染，否则可能出现错误结果。
6. 抗体溶液含有叠氮钠 NaN_3，需小心处理，不能食入，避免接触皮肤、黏膜、眼睛。例外，在酸性环境中，NaN_3 可形成危险物叠氮酸，因此试剂在丢弃之前，建议用大量的水稀释，以免有 NaN_3 的积聚和爆炸的危险。
7. 所有血标本要被当作潜在的感染源并小心处理（戴保护手套、护目镜、穿工作服）。
8. 禁止用嘴吸试剂和标本，避免皮肤、黏膜、眼睛和标本接触。
9. 采血管和一次性器材必须丢弃在用于焚烧的专用容器中。
10. 根据当地要求清理试剂和废物。

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【基本信息】

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【医疗器械备案凭证及技术要求编号】

国械备20181424号

【说明书批准日期及修改日期】

2018年10月11日

Pacific Blue™ anti-human/mouse Granzyme B Antibody

Catalog# / Size	515407 / 25 tests 515408 / 100 tests
Clone	GB11
Regulatory Status	RUO
Other Names	Granzyme-2, serine protease B, CCP1, Asp-ase, CTLA-1
Isotype	Mouse IgG1, κ
Description	Granzyme B is a 32 kD serine protease, also known as granzyme-2, serine protease B, CCP1, Asp-ase, and CTLA-1. Granzyme B is abundantly stored in the granules of cytotoxic T lymphocytes and NK cells. Low level of expression has been reported in granulocytes, B cells, and activated dendritic cells. Granzyme B is crucial for rapid induction of cell death and apoptosis through interaction with mannose-6-phosphate receptor.

Product Details

Verified Reactivity	Human, Mouse
Reported Reactivity	Rat
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with Pacific Blue™ under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	ICFC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. * Pacific Blue™ has a maximum emission of 455 nm when it is excited at 405 nm. Prior to using Pacific Blue™ conjugate for flow cytometric analysis, please verify your flow cytometer's capability of exciting and detecting the fluorochrome. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Violet Laser (405 nm)
Application References **(PubMed link indicates BioLegend citation)**	1. Wever PC, *et al.* 1998. *Immunology.* 93:383 2. Arens R, *et al.* 2004. *J. Exp. Med.* 199:1595 3. Lima M, *et al.* 2003. *Am. J. Pathol.* 163:763 4. Wiede F, *et al.* 2014. *J Autoimmun.* 53:105. PubMed 5. Baker GF, *et al.* 2014. *Cancer Res.* 74:5079. PubMed 6. Nacer A, *et al.* 2014. *PLoS Pathog.* 10:1004528. PubMed 7. Sharma SK, *et al.* 2015. *J Immunol.* 194:5529. PubMed
Product Citations	1. Moderzynski K, *et al.* 2016. PLoS Negl Trop Dis. . PubMed 2. Harsha Krovi S, *et al.* 2020. Nat Commun. 4.790277778. PubMed

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RRID

AB_2562195 (BioLegend Cat. No. 515407)
AB_2562196 (BioLegend Cat. No. 515408)

Antigen Details

Structure	32 kD serine protease
Distribution	Cytotoxic T-cells and NK cells, low on granulocytes, B cells and activated dendritic cells
Function	Induction of cell death and apoptosis
Ligand/Receptor	Mannose-6-phosphate receptor
Cell Type	B cells, Dendritic cells, NK cells, T cells
Biology Area	Cell Biology, Immunology, Innate Immunity, Neuroscience
Molecular Family	Enzymes and Regulators, Proteases
Antigen References	1. Estebanez-Perpina E, *et al.* 2000. *Biol Chem.* 381:1203 2. Griffiths GM. And S. Isaaz, *et al.* 1993. *J. Cell Biol.* 120:885 3. Spaeny-Dekking EH, *et al.* 1998. *J. Immunol.* 160:3610 4. Wagner C, *et al.* 2008. *Mol. Immunol.* 45:1761
Gene ID	3002 14939

171528

Related Protocols

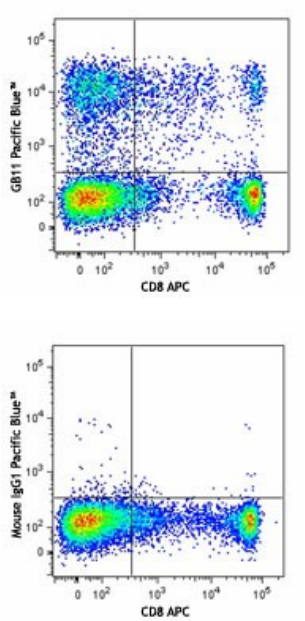
Surface and Intracellular Cytokine Staining for Flow Cytometry - Video

Intracellular Flow Cytometry Staining Protocol

Other Formats

FITC anti-human/mouse Granzyme B, Alexa Fluor® 647 anti-human/mouse Granzyme B, Pacific Blue™ anti-human/mouse Granzyme B

Product Data



Human peripheral blood lymphocytes were surface stained with CD8 APC and then intracellularly stained with Granzyme B (clone GB11) Pacific Blue™ (top) or mouse IgG1 Pacific Blue™ isotype control (bottom).

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PerCP/Cyanine5.5 anti-human/mouse Granzyme B Recombinant Antibody

Catalog# / Size	372211 / 25 tests 372212 / 100 tests
Clone	QA16A02
Regulatory Status	RUO
Other Names	Granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1, GZMB, CCP1, Asp-aseGranzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1, GZMB, CCP1, Asp-ase
Isotype	Mouse IgG1, κ
Description	Granzyme B is a 32 kD serine protease, also known as granzyme-2, serine protease B, CCP1, Asp-ase, and CTLA-1. Granzyme B is abundantly stored in the granules of cytotoxic T lymphocytes and NK cells. Low level of expression has been reported in granulocytes, B cells, and activated dendritic cells. Granzyme B is crucial for rapid induction of cell death and apoptosis through interaction with mannose-6-phosphate receptor.

Product Details

Verified Reactivity	Human, Mouse
Antibody Type	Recombinant
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with PerCP/Cyanine5.5 under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	ICFC - *Quality tested*
Recommended Usage	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume or 5 µL per 100 µL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application. * PerCP/Cyanine5.5 has a maximum absorption of 482 nm and a maximum emission of 690 nm.
Excitation Laser	Blue Laser (488 nm) Green Laser (532 nm)/Yellow-Green Laser (561 nm)
Additional Product Notes	BioLegend is in the process of converting the name PerCP/Cy5.5 to PerCP/Cyanine5.5. The dye molecule remains the same, so you should expect the same quality and performance from our PerCP/Cyanine5.5 products. Contact Technical Service if you have any questions.
Product Citations	1. Park SY, *et al.* 2022. NPJ Vaccines. 7:123. PubMed 2. Xiong A, *et al.* 2022. EBioMedicine. 83:104239. PubMed 3. Chen X, *et al.* 2021. Cell Rep. 37:109991. PubMed 4. Valestrand L, *et al.* 2022. Am J Pathol. . PubMed 5. Li Z, *et al.* 2022. Nat Commun. 13:6321. PubMed 6. Fang Y, *et al.* 2021. J Clin Invest. 131:00:00. PubMed 7. Montalban-Arques A, *et al.* 2021. Cell Host Microbe. :. PubMed 8. Prosser A, *et al.* 2021. STAR Protoc. 2:100810. PubMed 9. Han P, *et al.* 2020. Sci Adv. 6:eaaz1580. PubMed 10. Li CY, *et al.* 2022. Int J Mol Sci. 23:. PubMed
RRID	AB_2728378 (BioLegend Cat. No. 372211)

Antigen Details

Structure	32 kD serine protease
Distribution	Cytotoxic T cells, NK cells, and neutrophils, low on granulocytes, B cells and activated dendritic cells
Function	Granzyme B is able to induce target cell apoptosis by activating caspase independent pathways. Granzyme B is induced in $CD8^+$ T lymphocytes with ConA/ IL-2 and $CD4^+$ T lymphocytes with anti CD3/CD28 or CD3/CD46.
Interaction	Caspase-3
Ligand/Receptor	Mannose-6-phosphate receptor
Cell Type	T cells, NK cells, Neutrophils
Biology Area	Cell Biology, Immunology, Innate Immunity, Neuroscience
Molecular Family	Proteases, Enzymes and Regulators
Antigen References	1. Estebanez-Perpina E, *et al.* 2000. *Biol Chem.* 381:1203. 2. Griffiths GM. And S. Isaaz, *et al.* 1993. *J. Cell Biol.* 120:885. 3. Spaeny-Dekking EH, *et al.* 1998. *J. Immunol.* 160:3610. 4. Wagner C, *et al.* 2008. *Mol. Immunol.* 45:1761.
Gene ID	3002 14939

Related Protocols

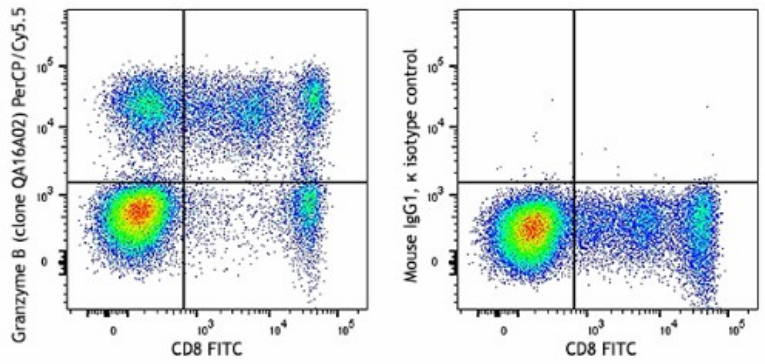
Surface and Intracellular Cytokine Staining for Flow Cytometry - Video

Intracellular Flow Cytometry Staining Protocol

Other Formats

Purified anti-human/mouse Granzyme B Recombinant Antibody, APC anti-human/mouse Granzyme B Recombinant Antibody, FITC anti-human/mouse Granzyme B Recombinant Antibody, PE anti-human/mouse Granzyme B Recombinant Antibody, PE/Cyanine7 anti-human/mouse Granzyme B Recombinant Antibody, Alexa Fluor® 700 anti-human/mouse Granzyme B Recombinant Antibody, Pacific Blue™ anti-human/mouse Granzyme B Recombinant Antibody, PerCP/Cyanine5.5 anti-human/mouse Granzyme B Recombinant Antibody, PE/Dazzle™ 594 anti-human/mouse Granzyme B Recombinant Antibody, Alexa Fluor® 647 anti-human/mouse Granzyme B Recombinant Antibody, APC/Fire™ 750 anti-human/mouse Granzyme B Recombinant Antibody, PE/Cyanine5 anti-human/mouse Granzyme B Recombinant Antibody

Product Data



Human peripheral blood mononuclear cells were stained with CD8 FITC, fixed, permeabilized, and then stained with Granzyme B (clone QA16A02, left) PerCP/Cyanine5.5 or mouse IgG1, κ PerCP/Cyanine5.5 isotype control (right).

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Annexin V-FITC/PI apoptosis kit (适用于除 C6 以外的流式细胞仪)

I. 产品信息

目 录 号： AP101 **规 格：** 30T, 60T, 100T

保　　存： 2-8℃避光，切勿冻存 **效 期：** 一年

储 存 液： 含0.09% NaN3 的磷酸盐缓冲液(pH7.2)

II. 背景简介

Annexin V(或Annexin A5)为胞内蛋白膜联蛋白家族成员，以钙依赖的方式与磷脂酰丝氨酸(PS)结合。PS 存在于正常细胞浆膜的内层，但在凋亡早期，膜不对称性丧失，PS 易位至细胞表面。荧光标记的 Annexin V 可与之特异性结合，表明该细胞为凋亡细胞。本试剂盒仅需 15 分钟即可染色，快速方便。

注：本产品不适用于自动调电压的流式细胞仪，如 BD 的 C6；如需用于此类仪器，推荐 Annexin V-FITC/PI apoptosis kit (C6 专用) (AP101C)。

III. 试剂盒组分

目录号 / 规格 / 组分	AP101-30	AP101-60	AP101-100
	30 T	60 T	100 T
Annexin V-FITC	150 µl	300 µl	500 µl
PI	300 µl	600 µl	1000 µl
5 × Binding buffer	5 ml	10 ml	15 ml
Apoptosis Positive Control Solution	5 ml	5 ml	5 ml

IV. 使用方法

一、 仪器参数调节

1. 收集 1×10^6 - 3×10^6 个细胞，用预冷 PBS 离心洗涤两次，弃上清。
2. 加入 500 µl Apoptosis Positive Control Solution 重悬，置冰上孵育 30 分钟。
3. 用预冷 PBS 离心洗涤，弃上清。
4. 加入适量预冷 1 × Binding Buffer 重悬，并加入数量相同且未经处理的活细胞与之混合。加入预冷 1 × Binding Buffer 补充至 1.5 ml，等分成三管，其中一管为空白对照管、两管为单染管。
5. 单染管分别加入 5 µl Annexin V-FITC 或10 µl PI，室温避光孵育 5 分钟。
6. 在流式细胞仪上，用空白管调节 FSC、SSC 和荧光通道的电压，并在此电压条件下，用单染管调节荧光通道的补偿。

注：某些将贴壁细胞处理为单个细胞的过程中会造成细胞膜损伤，从而造成 Annexin V 假阳性。因此需要进行优化。可使用对细胞更温和的酶如 Accutase 处理贴壁细胞。

二、 样本检测

1. 按实验方案诱导凋亡。

2. 用预冷 PBS 离心洗涤，收集 1 - 10 × 10^5 个细胞(包括培养上清中的细胞)。用双蒸水稀释 5 × Binding Buffer为 1 × 工作液，取 500 µl 1 × Binding Buffer 重悬细胞。

3. 每管加入 5 µl Annexin V-FITC 和10 µl PI。

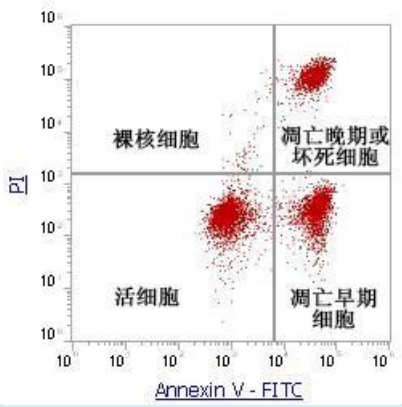
4. 轻柔涡旋混匀后，室温避光孵育 5 分钟。

5. 根据实验方法，进行流式分析

6. 流式分析

在流式细胞仪上，通过 FITC 检测通道检测 Annexin V-FITC (Ex = 488 nm; Em = 530 nm)和通过 PI 检测通道(Ex = 535 nm; Em = 615 nm)检测 PI。

V. 结果示例



VI. 注意事项

1. 请在使用本产品前仔细阅读说明书。本产品仅用于科研，不可用于诊断。

2. 为了您的安全和健康，请穿戴实验防护服、手套、口罩等必要的防护装备。

3. 更多凋亡相关产品敬请关注联科生物网站或来电咨询。

VII. 部分相关产品

目录号	产品名称	规格
AP101C-30	Annexin V-FITC/PI apoptosis kit (C6 专用)	30 T
AP104-30	Annexin V-PE/7-AAD apoptosis kit	30 T
AP105-30	Annexin V-APC/7-AAD apoptosis kit	30 T
AP107-30	Annexin V-APC/PI apoptosis kit	30 T
AP-30-PCS	Apoptosis Positive Control Solution	5 ml
CC01	胰蛋白酶-EDTA 消化液(含酚红)	100 ml
CC03	胰蛋白酶消化液(含酚红)	100 ml
CCS012	Cell cycle staining Kit	50 T
MJ101	Mitochondria Staining Kit (JC-1)	125 T

Technical Data Sheet

APC Annexin V

Product Information

Material Number:	**550474**
Size:	100 tests
Vol. per Test:	5 µl
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenence of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including APC. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis. Since externalization of PS occurs in the earlier stages of apoptosis, APC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

APC Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with APC Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (7-AAD negative, APC Annexin V positive). Viable cells with intact membranes exclude 7-AAD, whereas the membranes of dead and damaged cells are permeable to 7-AAD. For example, cells that are considered viable are both APC Annexin V and 7-AAD negative while cells that are in early apoptosis are APC Annexin V positive and 7-AAD negative, while cells that are in late apoptosis or already dead are both APC Annexin V and 7-AAD positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both APC Annexin V and 7-AAD. However, when apoptosis is measured over time, cells can be often tracked from APC Annexin V and 7-AAD negative (viable, or no measurable apoptosis), to APC Annexin V positive and 7-AAD negative (early apoptosis, membrane integrity is present) and finally to APC Annexin V and 7-AAD positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both APC Annexin V and 7-AAD positive, in of itself, reveals less information about the process by which the cells underwent their demise.

APC Annexin V is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

BD Biosciences

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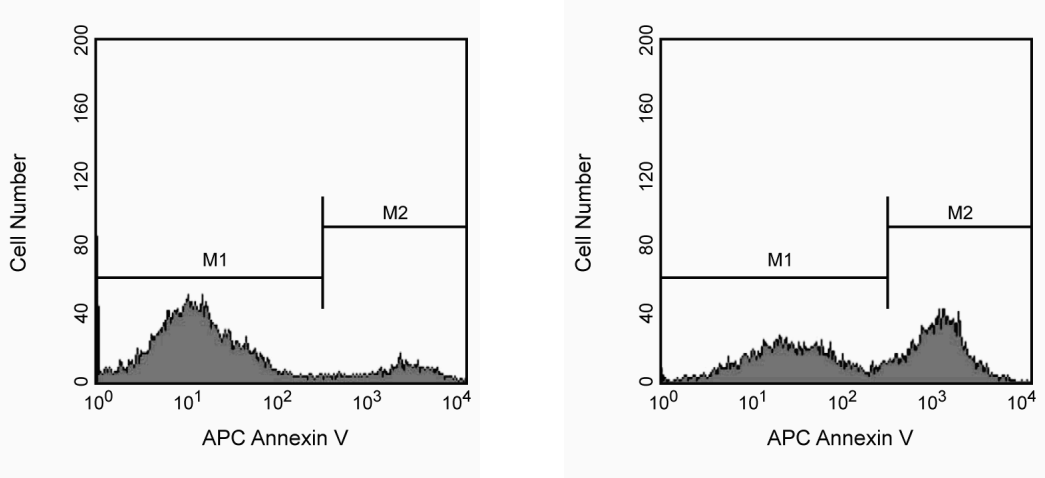
BD BD Biosciences



APC Annexin V: A tool for identifying cells that are undergoing apoptosis. *Jurkat T cells were left untreated (left panel) or treated for 4 hours (right panel) with 6 µM camptothecin. Cells were incubated with APC Annexin V and analyzed by flow cytometry. Untreated cells were primarily APC Annexin V negative, indicating that they were viable and not undergoing apoptosis. After a 4 hour treatment with camptothecin, there were two populations of cells: cells undergoing apoptosis (APC Annexin V positive), and cells that were viable and not undergoing apoptosis (APC Annexin V negative).*

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

APC Annexin V is a sensitive probe for identifying apoptotic cells, binding to negatively charged phospholipid surfaces (Kd of ~5 x 10e2) with a higher affinity for phosphatidylserine (PS) than most other phospholipids. APC Annexin V binding is calcium dependent and defined calcium and salt concentrations are required for optimal staining as described in the APC Annexin V Staining Protocol. **Investigators should note that APC Annexin V flow cytometric analysis on adherent cell types (e.g HeLa, NIH 3T3, etc.) is not routinely tested as specific membrane damage may occur during cell detachment or harvesting. Methods for utilizing Annexin V for flow cytometry on adherent cell types, however, have been previously reported (Casiola-Rosen et al. and van Engelend et al.).**

INDUCTION OF APOPTOSIS BY CAMPTOTHECIN

The following protocol is provided as an illustration on how APC Annexin V may be used on a cell line (Jurkat).

Materials

1. Prepare Camptothecin stock solution (Sigma-Aldrich Cat.No. C-9911): 1 mM in DMSO.
2. Jurkat T cells (ATCC TIB-152).

Procedure

1. Add Camptothecin (final conc. 4-6 µM) to 1 x 10e6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the APC Annexin V Staining Protocol to measure apoptosis.

APC ANNEXIN V STAINING PROTOCOL

Reagents

1. APC Annexin V: Included. Use 5 µl per test.
2. 7-Amino-Actinomycin D (7-AAD): Not included. 7-AAD (Cat.No. 559925) is a convenient, ready-to-use nucleic acid dye with fluorescence detectable in the far red range of the spectrum. Use 5 µl per test.
3. 10X Binding Buffer: Not Included. 0.1 M Hepes (pH 7.4) 1.4 M NaCl, 25 mM CaCl2. Store at 4°C. Alternatively, catalog number 556454 may be purchased.

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10e6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10e5 cells) to a 5 ml culture tube.
3. Add 5 µl of APC Annexin V (for one and two color analysis) and 5 µl of 7-AAD (for two color analysis only).
4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

The following controls are used to set up compensation and quadrants:

1. Unstained cells.
2. Cells stained with APC Annexin V alone (no 7-AAD).
3. Cells stained with 7-AAD alone (no APC Annexin V).

Other Staining Controls

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with APC Annexin V and/or APC Annexin V and 7-AAD. It is important to note that the basal level of apoptosis and necrosis varies considerably within a population. Thus, even in the absence of induced apoptosis, most cell populations will contain a minor percentage of cells that are positive for apoptosis (APC Annexin V positive, 7-AAD negative or APC Annexin V positive, 7-AAD positive).

The untreated population is used to define the basal level of apoptotic and dead cells. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for 7-AAD as well as for APC Annexin V. Thus, the assay does not distinguish between cells that have already undergone an apoptotic cell death and those that have died as a result of necrotic pathway, because in either case the dead cells will stain with both APC Annexin V and 7-AAD.

Suggested Companion Products

Catalog Number	Name	Size	Clone
556454	Annexin V Binding Buffer, 10X concentrate	50 ml	(none)
559925	7-AAD	2.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.

References

Andree HA, Reutelingsperger CP, Hauptmann R, Hemker HC, Hermens WT, Willems GM. Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers. *J Biol Chem.* 1990; 265(9):4923-4928.(Biology)

Casciola-Rosen L, Rosen A, Petri M, Schlissel M. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus. *Proc Natl Acad Sci U S A.* 1996; 93(4):1624-1629.(Methodology: Apoptosis, Flow cytometry)

Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. *Blood.* 1995; 85(2):532-540.(Biology)

Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood.* 1994; 84(5):1415-1420.(Methodology: Apoptosis, Flow cytometry)

Martin SJ, Reutelingsperger CP, McGahon AJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med.* 1995; 182(5):1545-1556.(Biology)

Raynal P, Pollard HB. Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins. *Biochim Biophys Acta.* 1994; 1197(1):63-93.(Biology)

van Engeland M, Ramaekers FC, Schutte B, Reutelingsperger CP. A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. *Cytometry.* 1996; 24(2):131-139.(Methodology: Apoptosis, Flow cytometry)

Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J Immunol Methods.* 1995; 184(1):39-51.(Methodology: Apoptosis, Flow cytometry)

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Myc-Tag Rabbit mAb

Catalog No.: AE070 Recombinant 9 Publications

Basic Information

Observed MW
58KDa(NLK)/72KDa(YAP1)/55KDa(PTEN)

Calculated MW

Category
Tag antibody

Applications
WB,IF/ICC,FC,IP

Cross-Reactivity

CloneNo number
ARC5004-12

Background

Protein tags are peptide sequences genetically grafted onto a recombinant protein. Often these tags are removable by chemical agents or by enzymatic means, such as proteolysis or intein splicing. Tags are attached to proteins for various purposes.Epitope tags are short peptide sequences which are chosen because high-affinity antibodies can be reliably produced in many different species. These are usually derived from viral genes, which explain their high immunoreactivity. Epitope tags include V5-tag, Myc-tag, HA-tag and NE-tag. These tags are particularly useful for western blotting, immunofluorescence and immunoprecipitation experiments, although they also find use in antibody purification.

Recommended Dilutions

WB	1:5000-1:20000
IF/ICC	1:50 - 1:200
FC	1:50 - 1:200
IP	1:500-1:1000

Immunogen Information

Gene ID

Swiss Prot

Immunogen
A synthetic peptide corresponding to Myc tag.

Synonyms
Myc;Myc tag;Myc-tag

Contact

400-999-6126

cn.market@abclonal.com.cn

www.abclonal.com.cn

Product Information

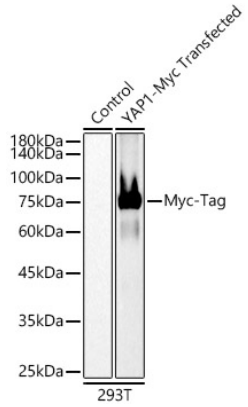
Source
Rabbit

Isotype
IgG

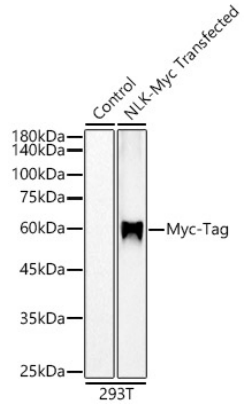
Purification
Affinity purification

Storage
Store at -20℃. Avoid freeze / thaw cycles.
Buffer: PBS with 0.05% proclin300,0.05% BSA,50% glycerol,pH7.3.

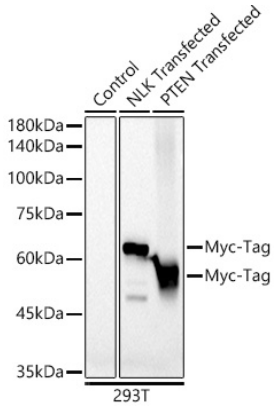
Validation Data



Western blot analysis of extracts of normal 293T cells, 293T transfected with YAP1 Protein, using Myc-Tag Rabbit mAb antibody (AE070) at 1:120000 dilution.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 1s.

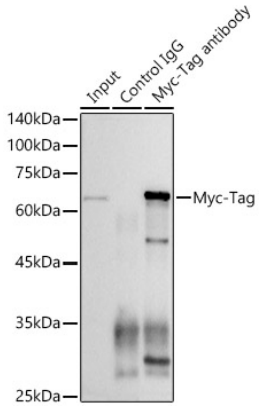


Western blot analysis of extracts of normal 293T cells, 293T transfected with NLK Protein, using Myc-Tag Rabbit mAb antibody (AE070) at 1:120000 dilution.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 10s.

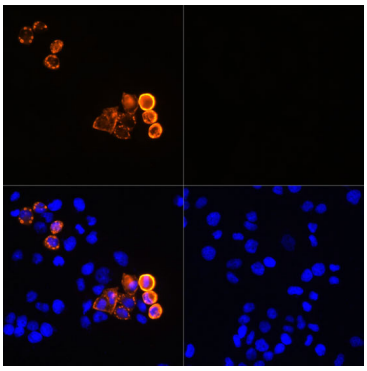


Western blot analysis of extracts of normal 293T cells,293T transfected with NLK Protein and 293T transfected with PTEN Protein, using Myc-Tag antibody (AE070) at 1:20000 dilution.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 10s.

Validation Data



Immunoprecipitation analysis of 300ug extract cell lysate from 293T cells transfected with NLK expression vector containing a Myc-Tag with 3ug Myc-Tag Rabbit mAb antibody（AE070).Western blot was performed from the immunoprecipitate using Myc-Tag Rabbit mAb antibody（AE070) at 1:5000 dilution.



Immunofluorescence analysis of HeLa cells transfected with Myc-Tag and untreated HeLa cells use Rabbit anti Myc-Tag mAb(AE070) at dilution of 1:100. Blue: DAPI for nuclear staining.

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Mouse anti HA-Tag mAb

Catalog No.: AE008 **77 Publications**

Basic Information

Observed MW
Refer to Figures

Calculated MW

Category
Tag antibody

Applications
WB,IF/ICC,IP

Cross-Reactivity

CloneNo number
AMC0503

Background

Protein tags are peptide sequences genetically grafted onto a recombinant protein. Often these tags are removable by chemical agents or by enzymatic means, such as proteolysis or intein splicing. Tags are attached to proteins for various purposes.Epitope tags are short peptide sequences which are chosen because high-affinity antibodies can be reliably produced in many different species. These are usually derived from viral genes, which explain their high immunoreactivity. Epitope tags include V5-tag, Myc-tag, HA-tag and NE-tag. These tags are particularly useful for western blotting, immunofluorescence and immunoprecipitation experiments, although they also find use in antibody purification.

Recommended Dilutions

WB	1:2000 - 1:5000
IF/ICC	1:50 - 1:500
IP	1:20 - 1:50

Immunogen Information

Gene ID

Swiss Prot

Immunogen
A synthetic peptide corresponding to HA tag.

Synonyms
HA;HA tag;HA-tag

Contact

400-999-6126

cn.market@abclonal.com.cn

www.abclonal.com.cn

Product Information

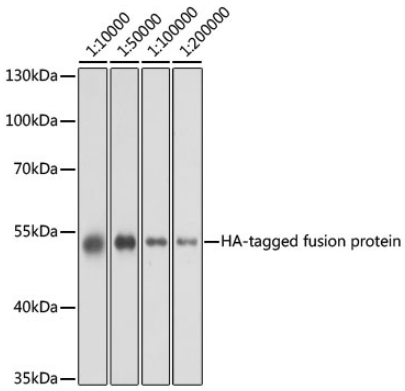
Source
Mouse

Isotype
IgG1,Kappa

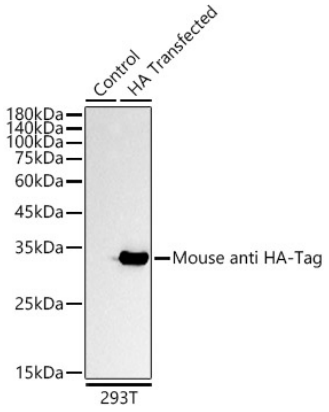
Purification
Affinity purification

Storage
Store at -20℃. Avoid freeze / thaw cycles.
Buffer: PBS with 0.01% thiomersal,50% glycerol,pH7.3.

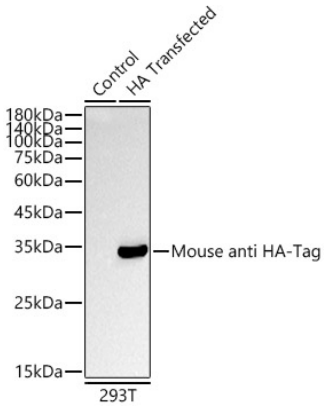
Validation Data



Western blot analysis of over-expressed HA-tagged protein in 293T cell using HA-tag antibody (AE008) at different dilution. Each lane was loaded with 2 ug cell lysate.
Secondary antibody: HRP Goat Anti-Mouse IgG (H+L) (AS003) at 1:10000 dilution.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 1s.

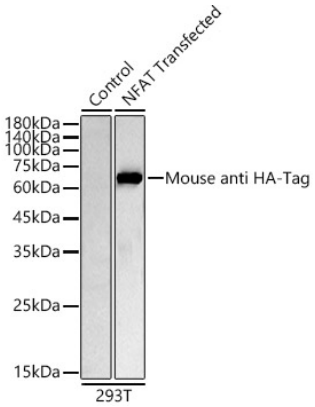


Western blot analysis of extracts of 293T cells, using Mouse anti HA-Tag mAb antibody (AE008) at 1:5000 dilution.
Secondary antibody: HRP Goat Anti-Mouse IgG (H+L) (AS003) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 10s.

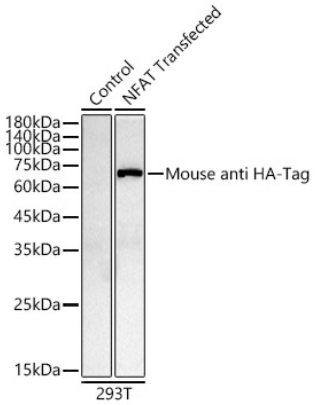


Western blot analysis of extracts of 293T cells, using Mouse anti HA-Tag mAb antibody (AE008) at 1:10000 dilution.
Secondary antibody: HRP Goat Anti-Mouse IgG (H+L) (AS003) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 10s.

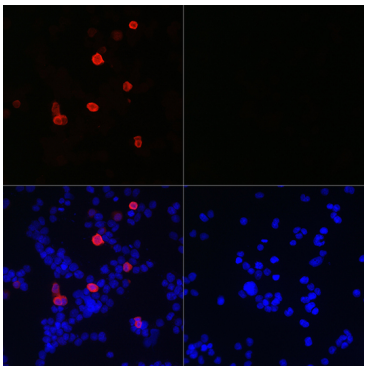
Validation Data



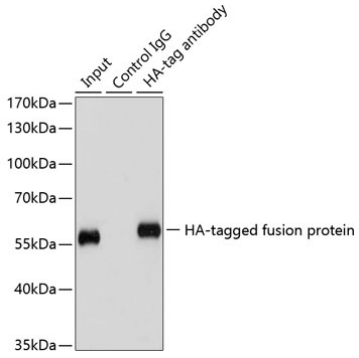
Western blot analysis of extracts of 293T cells, using Mouse anti HA-Tag mAb antibody (AE008) at 1:5000 dilution.
Secondary antibody: HRP Goat Anti-Mouse IgG (H+L) (AS003) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 180s.



Western blot analysis of extracts of 293T cells, using Mouse anti HA-Tag mAb antibody (AE008) at 1:10000 dilution.
Secondary antibody: HRP Goat Anti-Mouse IgG (H+L) (AS003) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 180s.



Immunofluorescence analysis of 293T cells transfected with HA-Tag fusion protein and untreated 293T cells use Mouse anti HA-Tag mAb (AE008) at dilution of 1:50 (40x lens). Blue: DAPI for nuclear staining.



Immunoprecipitation of over-expressed HA-tagged protein in 293T cells using HA-tag antibody (AE008). A mock served as negative control and over-expressed 293T cell lysate served as positive control.

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Vinculin Rabbit mAb

Catalog No.: A2752 Recombinant 4 Publications

Basic Information

Observed MW
124kDa

Calculated MW
125kDa

Category
Primary antibody

Applications
WB,IF/ICC,IP

Cross-Reactivity
Human, Mouse, Rat

CloneNo number
ARC51900

Background

Vinculin is a cytoskeletal protein associated with cell-cell and cell-matrix junctions, where it is thought to function as one of several interacting proteins involved in anchoring F-actin to the membrane. Defects in VCL are the cause of cardiomyopathy dilated type 1W. Dilated cardiomyopathy is a disorder characterized by ventricular dilation and impaired systolic function, resulting in congestive heart failure and arrhythmia. Multiple alternatively spliced transcript variants have been found for this gene, but the biological validity of some variants has not been determined. [provided by RefSeq, Jul 2008]

Recommended Dilutions

WB	1:500 - 1:2000
IF/ICC	1:50 - 1:200
IP	1:50 - 1:200

Immunogen Information

Gene ID
7414

Swiss Prot
P18206

Immunogen
A synthetic peptide corresponding to a sequence within amino acids 800-900 of human Vinculin (NP_054706.1).

Synonyms
CMD1W; CMH15; HEL114; MV; MVCL

Contact

400-999-6126

cn.market@abclonal.com.cn

www.abclonal.com.cn

Product Information

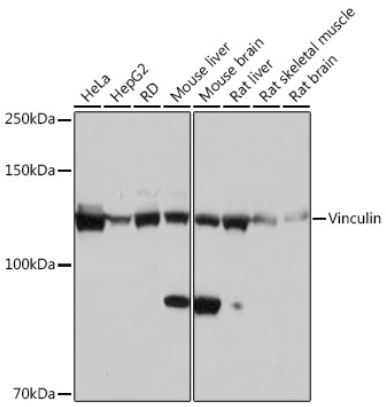
Source
Rabbit

Isotype
IgG

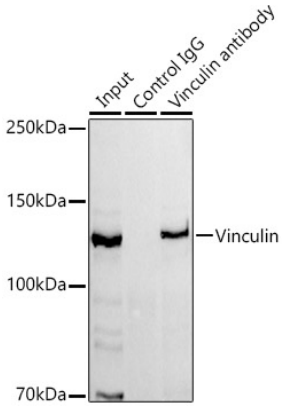
Purification
Affinity purification

Storage
Store at -20℃. Avoid freeze / thaw cycles.
Buffer: PBS with 0.05% proclin300,0.05% BSA,50% glycerol,pH7.3.

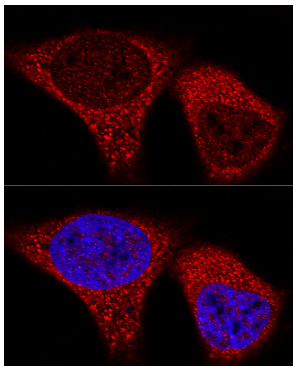
Validation Data



Western blot analysis of extracts of various cell lines, using Vinculin Rabbit mAb (A2752) at 1□1000 dilution.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 1s.



Immunoprecipitation analysis of 300ug extracts of HeLa cells using 3ug Vinculin antibody (A2752).
Western blot was performed from the immunoprecipitate using Vinculin antibody (A2752) at a dilution of 1:1000.



Confocal imaging of HeLa cells using Vinculin Rabbit mAb (A2752, dilution 1:100) (Red). DAPI was used for nuclear staining (blue). Objective: 60x.

Revision 4

Store at -20C

#4967

β-Actin Antibody

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, W-S	H M R Hm Mk Mi Dm Z B	Endogenous	45	Rabbit	P60709	60

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

β-Actin Antibody detects endogenous levels of β-actin. Due to the high sequence identity between the actin isoforms, β-Actin Antibody may cross-react with other actin isoforms.

Species Reactivity:
Human, Mouse, Rat, Hamster, Monkey, Mink, D. melanogaster, Zebrafish, Bovine

Species predicted to react based on 100% sequence homology:
Chicken, Xenopus, Dog, Pig, Horse

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues of human β-actin. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Actin, a ubiquitous eukaryotic protein, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β- and γ-actin, also known as cytoplasmic actin, are ubiquitously expressed, controlling cell structure and motility (1). While all actin isoforms are highly homologous, cytoplasmic β- and γ-actin protein sequences differ by only four biochemically similar amino acids (2). For this reason, antibodies raised to β-actin may cross-react with γ-actin, and vice versa. α-cardiac and α-skeletal actin are expressed in striated cardiac and skeletal muscles, respectively; two smooth muscle actins, α- and γ-actin, are found primarily in vascular smooth muscle and enteric smooth muscle, respectively. These actin isoforms regulate the contractile potential of muscle cells (1). Actin exists mainly as a fibrous polymer, F-actin. In response to cytoskeletal reorganizing signals during processes such as cytokinesis, endocytosis, or stress, cofilin promotes fragmentation and depolymerization of F-actin, resulting in an increase in the monomeric globular form, G-actin (3). The ARP2/3 complex stabilizes F-actin fragments and promotes formation of new actin filaments (3). Research studies have shown that actin is hyperphosphorylated in primary breast tumors (4). Cleavage of actin under apoptotic conditions has been observed *in vitro* and in cardiac and skeletal muscle, as shown in research studies (5-7). Actin cleavage by caspase-3 may accelerate ubiquitin/proteasome-dependent muscle proteolysis (7).

1. Herman, I.M. (1993) *Curr. Opin. Cell Biol.* 5, 48-55.
2. Perrin, B.J. and Ervasti, J.M. (2010) *Cytoskeleton (Hoboken)* 67, 630-4.
3. Condeelis, J. (2001) *Trends Cell Biol* 11, 288-93.
4. Lim, Y.P. et al. (2004) *Clin Cancer Res* 10, 3980-7.
5. Kayalar, C. et al. (1996) *Proc Natl Acad Sci U S A* 93, 2234-8.
6. Communal, C. et al. (2002) *Proc Natl Acad Sci U S A* 99, 6252-6.
7. Du, J. et al. (2004) *J Clin Invest* 113, 115-23.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

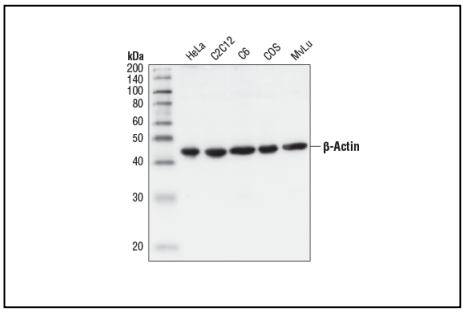
APPLICATIONS KEY **WB:** Western Blotting **W-S:** Simple Western™

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

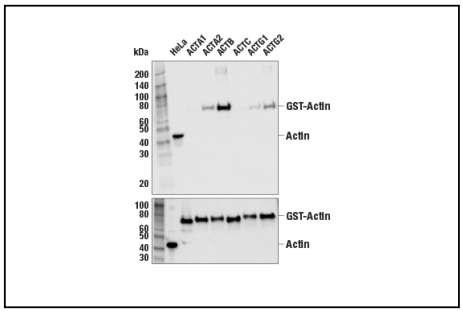
Revision 4

#4967

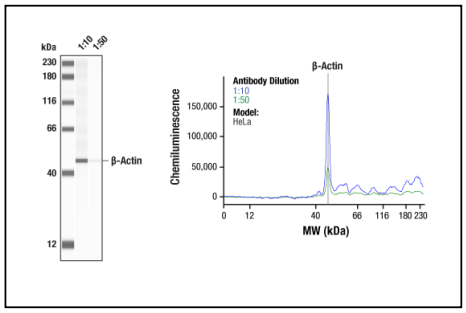
β-Actin Antibody



Western blot analysis of extracts from HeLa, C2C12, C6, COS, and MvLu cells, using β-Actin Antibody.



Western blot analysis of recombinant Actin isoforms using β-Actin Antibody (upper) and Pan-Actin Antibody #4968 (lower).



Simple Western™ analysis of lysates (1 mg/mL) from HeLa cells using ß-Actin Antibody #4967. The virtual lane view (left) shows the target band (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.

#4967

β-Actin Antibody

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Revision 1

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#9211

Phospho-p38 MAPK (Thr180/Tyr182) Antibody

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC	H M R Mk Dm Pg Sc	Endogenous	43	Rabbit	Q16539, O15264, P53778, Q15759	1432, 5603, 6300, 5600

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (Immunocytochemistry)	1:400

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Phospho-p38 MAPK (Thr180/Tyr182) Antibody detects endogenous levels of p38 MAPK only when activated by phosphorylation at threonine 180 and tyrosine 182. This antibody does not cross-react with the phosphorylated forms of either p42/44 MAPK or SAPK/JNK. It will also react with p38 singly phosphorylated at Thr180 and singly phosphorylated at Tyr182.

Species Reactivity:

Human, Mouse, Rat, Monkey, D. melanogaster, Pig, S. cerevisiae

Species predicted to react based on 100% sequence homology:

Hamster, Zebrafish, Bovine

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Thr180/Tyr182 of human p38 MAPK. Antibodies are purified by protein A and peptide affinity chromatography.

Background

p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase that participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAPK, p38α, β, γ (also known as Erk6 or SAPK3), and δ (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAPK is activated by a variety of cellular stresses, including osmotic shock, inflammatory cytokines, lipopolysaccharide (LPS), UV light, and growth factors (1-5). MKK3, MKK6, and SEK activate p38 MAPK by phosphorylation at Thr180 and Tyr182. Activated p38 MAPK has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6), and MEF2 (5-8). SB203580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-imidazole) is a selective inhibitor of p38 MAPK. This compound inhibits the activation of MAPKAPK-2 by p38 MAPK and subsequent phosphorylation of HSP27 (9). SB203580 inhibits p38 MAPK catalytic activity by binding to the ATP-binding pocket, but does not inhibit phosphorylation of p38 MAPK by upstream kinases (10).

1. Rouse, J. et al. (1994) *Cell* 78, 1027-37.
2. Han, J. et al. (1994) *Science* 265, 808-11.
3. Lee, J.C. et al. (1994) *Nature* 372, 739-46.
4. Freshney, N.W. et al. (1994) *Cell* 78, 1039-49.
5. Raingeaud, J. et al. (1995) *J Biol Chem* 270, 7420-6.
6. Zervos, A.S. et al. (1995) *Proc Natl Acad Sci U S A* 92, 10531-4.
7. Zhao, M. et al. (1999) *Mol Cell Biol* 19, 21-30.
8. Yang, S.H. et al. (1999) *Mol Cell Biol* 19, 4028-38.
9. Cuenda, A. et al. (1995) *FEBS Lett* 364, 229-33.
10. Kumar, S. et al. (1999) *Biochem Biophys Res Commun* 263, 825-31.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

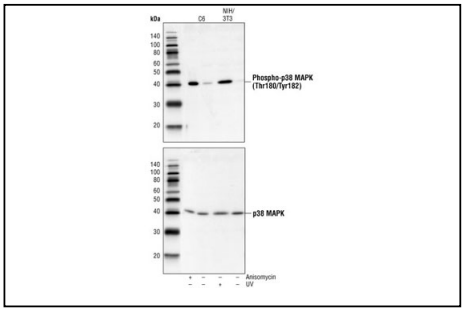
APPLICATIONS KEY **WB:** Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

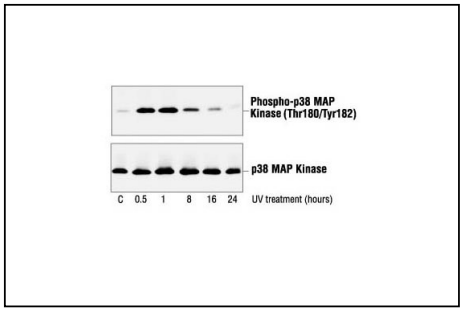
Revision 1

#9211

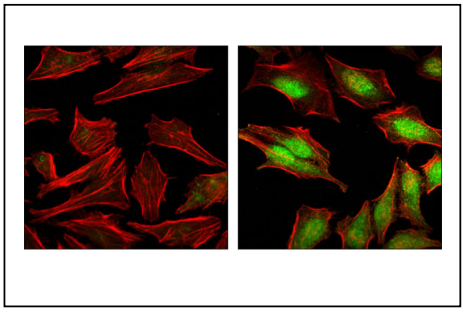
Phospho-p38 MAPK (Thr180/Tyr182) Antibody



Western blot analysis of extracts from C6 cells, untreated or anisomycin-treated, and NIH/3T3 cells, untreated or UV-treated, using Phospho-p38 MAPK (Thr180/Tyr182) Antibody (upper) or p38 MAPK Antibody #9212 (lower).



Western blot analysis of extracts from UV-treated NIH/3T3 cells, using Phospho-p38 MAPK (Thr180/Tyr182) Antibody (upper) or control p38 MAPK Antibody #9212 (lower).



Confocal immunofluorescent analysis of HeLa cells -/+ UV light, labeled with Phospho-p38 MAPK (green). Absence of staining in untreated cells (left) and nuclear localization in treated cells (right). Red = Actin filaments (phalloidin).

#9211

Phospho-p38 MAPK (Thr180/Tyr182) Antibody

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Revision 5

Store at -20C

#8690

p38 MAPK (D13E1) XP® Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IHC-P, IF-IC, FC-FP	H M R Hm Mk B Pg	Endogenous	40	Rabbit IgG	Q16539, P53778, Q15759	1432, 6300, 5600

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:200 - 1:800
Immunofluorescence (Immunocytochemistry)	1:100 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:200 - 1:800

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #54470.

Specificity / Sensitivity

p38 MAPK (D13E1) XP® Rabbit mAb recognizes endogenous levels of total p38α, β, or γ MAPK protein. This antibody does not recognize p38δ, SAPK/JNK, or p44/42 MAPK proteins.

Species Reactivity:

Human, Mouse, Rat, Hamster, Monkey, Bovine, Pig

Species predicted to react based on 100% sequence homology:

Chicken

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human p38 protein.

Background

p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase that participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAPK, p38α, β, γ (also known as Erk6 or SAPK3), and δ (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAPK is activated by a variety of cellular stresses, including osmotic shock, inflammatory cytokines, lipopolysaccharide (LPS), UV light, and growth factors (1-5). MKK3, MKK6, and SEK activate p38 MAPK by phosphorylation at Thr180 and Tyr182. Activated p38 MAPK has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6), and MEF2 (5-8). SB203580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-imidazole) is a selective inhibitor of p38 MAPK. This compound inhibits the activation of MAPKAPK-2 by p38 MAPK and subsequent phosphorylation of HSP27 (9). SB203580 inhibits p38 MAPK catalytic activity by binding to the ATP-binding pocket, but does not inhibit phosphorylation of p38 MAPK by upstream kinases (10).

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2. Han, J. et al. (1994) *Science* 265, 808-11.
3. Lee, J.C. et al. (1994) *Nature* 372, 739-46.
4. Freshney, N.W. et al. (1994) *Cell* 78, 1039-49.
5. Raingeaud, J. et al. (1995) *J Biol Chem* 270, 7420-6.
6. Zervos, A.S. et al. (1995) *Proc Natl Acad Sci U S A* 92, 10531-4.
7. Zhao, M. et al. (1999) *Mol Cell Biol* 19, 21-30.
8. Yang, S.H. et al. (1999) *Mol Cell Biol* 19, 4028-38.
9. Cuenda, A. et al. (1995) *FEBS Lett* 364, 229-33.
10. Kumar, S. et al. (1999) *Biochem Biophys Res Commun* 263, 825-31.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

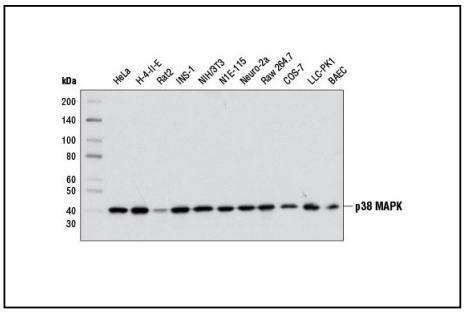
APPLICATIONS KEY **WB:** Western Blotting **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

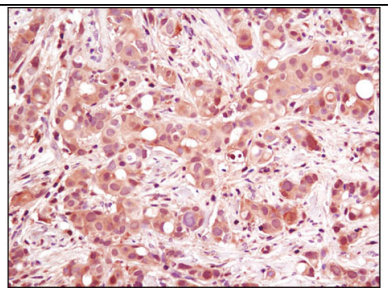
Revision 5

#8690

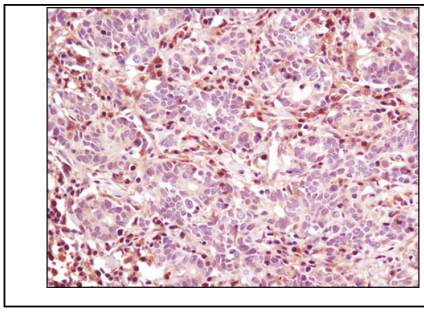
p38 MAPK (D13E1) XP® Rabbit mAb



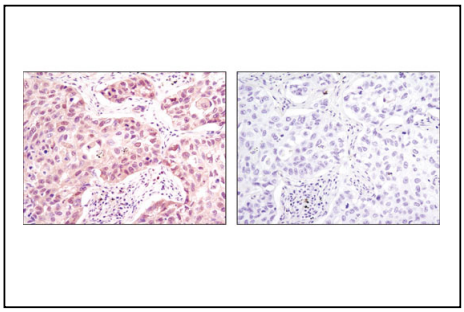
Western blot analysis of extracts from various cell lines using p38 MAPK (D13E1) XP® Rabbit mAb.



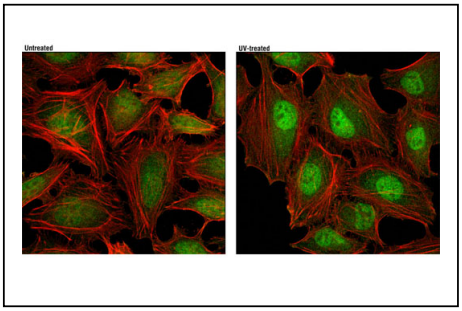
Immunohistochemical analysis of paraffin-embedded human breast carcinoma using p38 MAPK (D13E1) XP® Rabbit mAb.



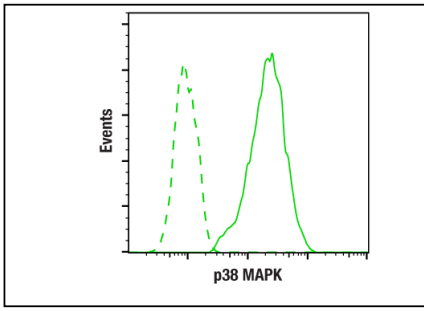
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using p38 MAPK (D13E1) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using p38 MAPK (D13E1) XP® Rabbit mAb in the presence of control peptide (left) or antigen-specific peptide (right).



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or treated with UV (100 mJ/cm^2 with 30 min recovery; right), using p38 MAPK (D13E1) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).



Flow cytometric analysis of HeLa cells using p38 MAPK (D13E1) XP® Rabbit mAb (solid line) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype control #3900 (dashed line). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

#8690

p38 MAPK (D13E1) XP® Rabbit mAb

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Revision 7

Store at -20C

#2855

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IHC-P, IF-IC, FC-FP	H M R Mk Dm	Endogenous	15 to 20	Rabbit IgG	Q13541	1978

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:800 - 1:3200
Immunofluorescence (Immunocytochemistry)	1:200 - 1:800
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #39788.

Specificity / Sensitivity

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb detects endogenous levels of 4E-BP1 only when phosphorylated at Thr37 and/or Thr46. This antibody may cross-react with 4E-BP2 and 4E-BP3 when phosphorylated at equivalent sites. Non-specific staining has been observed in mitotic cells by immunofluorescence.

Species Reactivity:
Human, Mouse, Rat, Monkey, D. melanogaster

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr37 and Thr46 of mouse 4E-BP1.

Background

Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the translation initiation factor eIF4E. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR at Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

1. Pause, A. et al. (1994) *Nature* 371, 762-7.
2. Brunn, G.J. et al. (1997) *Science* 277, 99-101.
3. Gingras, A.C. et al. (1998) *Genes Dev* 12, 502-13.
4. Fadden, P. et al. (1997) *J Biol Chem* 272, 10240-7.
5. Gingras, A.C. et al. (1999) *Genes Dev* 13, 1422-37.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

APPLICATIONS KEY **WB:** Western Blotting **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

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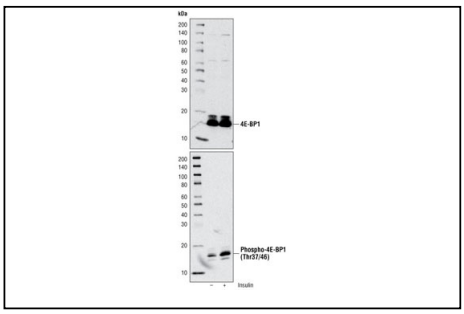
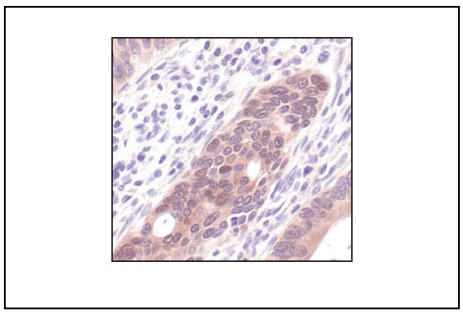
Revision 7

#2855

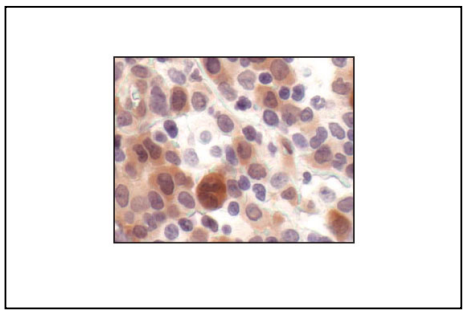
Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb



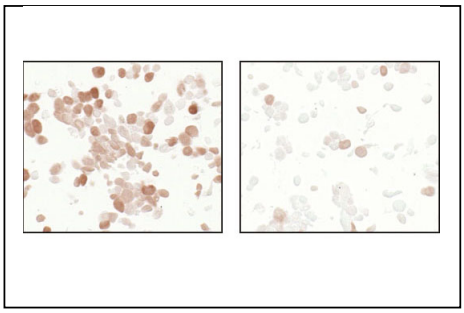
Western blot analysis of extracts from 293T cells using 4E-BP1 Antibody #9452 (upper) and Phospho-4E-BP1 (Thr37/46) Antibody #2855 (lower). The cells were starved for 24 hours in serum-free medium and underwent a 1 hour amino acid deprivation. Amino acids were replenished for 1 hour. Cells were then either untreated (-) or treated with 100 nM insulin (+) for 30 minutes.



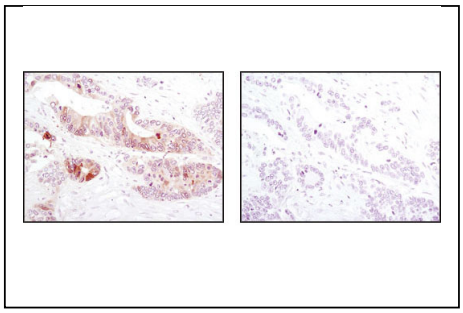
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



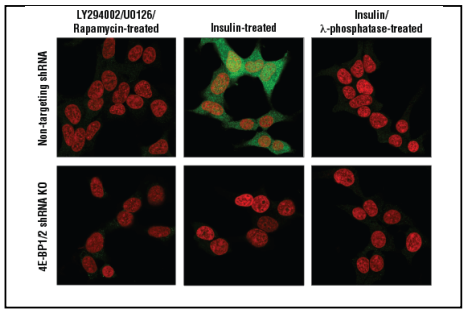
Immunohistochemical analysis of paraffin-embedded human lymphoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (left) or LY294002-treated (right), using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb on SignalSlide (TM) Phospho-Akt (Ser473) IHC Controls #8101.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb in the presence of control peptide (left) or Phospho-4E-BP1 (Thr37/46) Blocking Peptide #1052 (right).

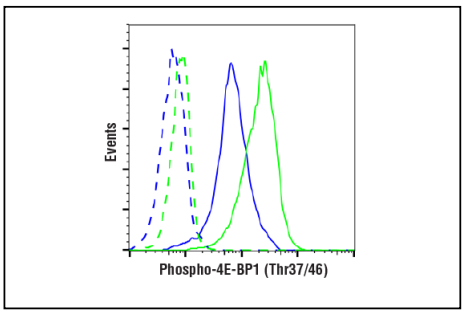


Confocal immunofluorescent analysis of 293 cells, expressing either non-targeting shRNA (top) or shRNA targeting 4E-BP1/2 (bottom), using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (green). To confirm phospho-specificity, cells were treated with an inhibitor cocktail consisting of LY294002 #9901, U0126 #9903, and Rapamycin #9904 (50 µM; 10 µm; 10 nM; 2 hr) (left), stimulated with insulin (100 nM, 30 min; middle), or processed with λ-phosphatase following insulin stimulation (right). Red = Propidium Iodide (PI)/RNase Staining Solution (#4087).

Revision 7

#2855

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb



Flow cytometric analysis of Jurkat cells, untreated (green) or treated with LY294002 #9901, Wortmannin #9951, and U0126 #9903 (50 µM, 1 µM, and 10 µM, 2 hr; blue) using Phospho-4E-BP1 (Thr36/46) (236B4) Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

#2855

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb

限制使用

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Revision 5

Store at -20C

#9644

4E-BP1 (53H11) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC, FC-FP	H M R Mk	Endogenous	15-20	Rabbit IgG	Q13541	1978

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:1200 - 1:4800
Immunofluorescence (Immunocytochemistry)	1:800 - 1:3200
Flow Cytometry (Fixed/Permeabilized)	1:1600

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #42235.

Specificity / Sensitivity

4E-BP1 (53H11) Rabbit mAb detects endogenous levels of total 4E-BP1 protein.

Species Reactivity:
Human, Mouse, Rat, Monkey

Source / Purification

4E-BP1 (53H11) Rabbit mAb is produced by immunizing rabbits with a synthetic peptide corresponding to residues surrounding Ser112 of human 4E-BP1.

Background

Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the translation initiation factor eIF4E. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR at Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

1. Pause, A. et al. (1994) *Nature* 371, 762-7.
2. Brunn, G.J. et al. (1997) *Science* 277, 99-101.
3. Gingras, A.C. et al. (1998) *Genes Dev* 12, 502-13.
4. Fadden, P. et al. (1997) *J Biol Chem* 272, 10240-7.
5. Gingras, A.C. et al. (1999) *Genes Dev* 13, 1422-37.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

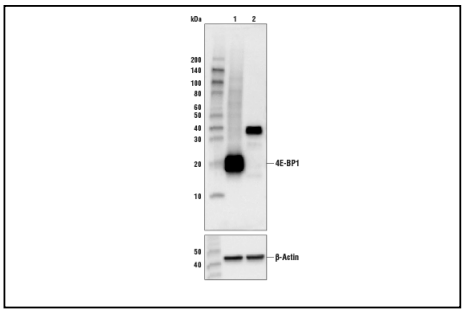
APPLICATIONS KEY **WB:** Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

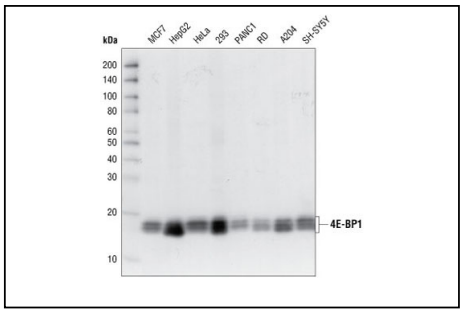
Revision 5

#9644

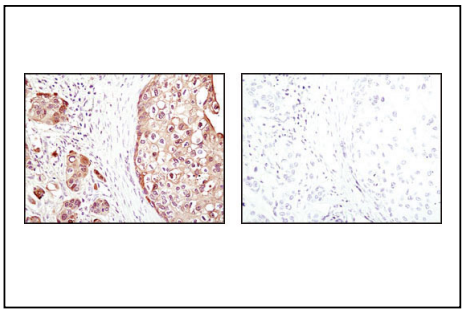
4E-BP1 (53H11) Rabbit mAb



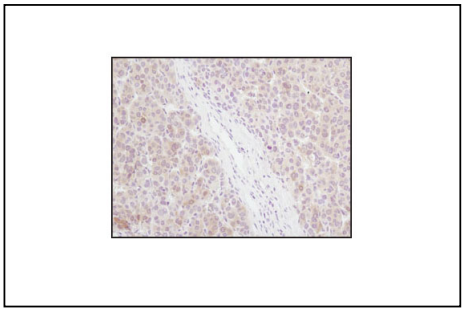
Western blot analysis of extracts from control HeLa cells (Lane 1) or HeLa cells with a targeted mutation in the gene encoding 4E-BP1 (Lane 2) using 4E-BP1 (53H11) Rabbit mAb (upper) or β-actin (13E5) Rabbit mAb #4970 (lower). The change in 4E-BP1 molecular weight in the mutated HeLa cells confirms the specificity of the antibody for 4E-BP1.



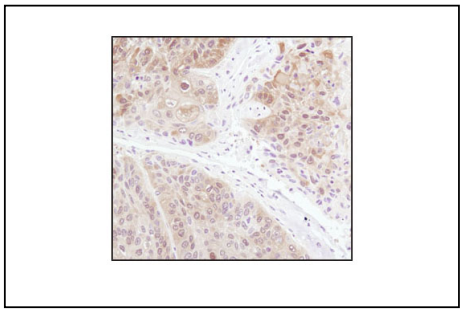
Western blot analysis of extracts from various cell lines using 4E-BP1 (53H11) Rabbit mAb.



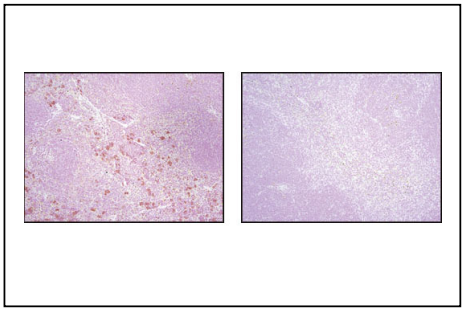
Immunohistochemical analysis of paraffin-embedded human breast carcinoma using 4E-BP1 (53H11) Rabbit mAb in the presence of control peptide (left) or 4E-BP1 blocking peptide #1053 (right).



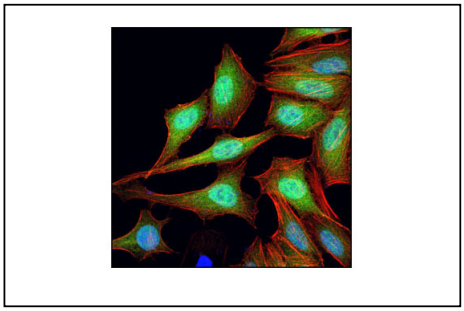
Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma using 4E-BP1 (53H11) Rabbit mAb.



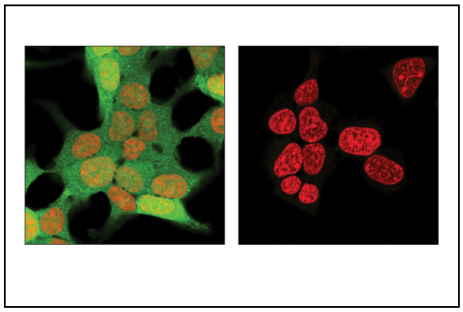
Immunohistochemical analysis of paraffin-embedded human lung carcinoma using 4E-BP1 (53H11) Rabbit mAb.



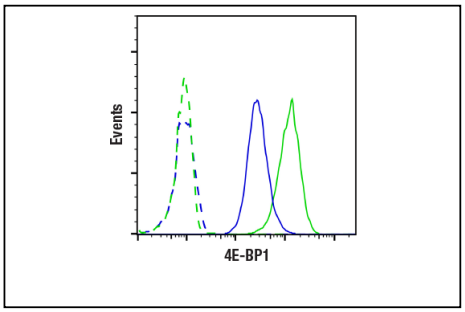
Immunohistochemical analysis of paraffin-embedded mouse spleen, 4E-BP1/2 wild type (left) or 4E-BP1 knockout (right), using 4E-BP1 (53H11) Rabbit mAb. 4E-BP wild type and knockout tissues kindly provided by Dr. Nahum Sonenberg, McGill University.



Confocal immunofluorescent analysis of HeLa cells using 4E-BP1 (53H11) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Confocal immunofluorescent analysis of 293 cells, expressing either nontargeting shRNA (left) or shRNA targeting 4E-BP1/2 (right), using 4E-BP1 (53H11) Rabbit mAb #9644 (green). Red = Propidium Iodide (PI)/RNase Staining Solution #4084.



Flow cytometric analysis of 293 cells, transfected with control shRNA (green) or 4E-BP1-specific shRNA (blue) using 4E-BP1 (53H11) Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

#9644

4E-BP1 (53H11) Rabbit mAb

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Revision 5

Store at -20C

#3033

Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IP, IF-IC, FC-FP	H M R Hm Mk Pg	Endogenous	65	Rabbit IgG	Q04206	5970

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:50
Immunofluorescence (Immunocytochemistry)	1:800 - 1:3200
Flow Cytometry (Fixed/Permeabilized)	1:800 - 1:3200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #76778.

Specificity / Sensitivity

Phospho-NF-kappaB p65 (Ser536) (93H1) Rabbit mAb detects NF-κB p65 only when phosphorylated at Ser536. It does not cross-react with the p50 subunit or other related proteins.

Species Reactivity:
Human, Mouse, Rat, Hamster, Monkey, Pig

Species predicted to react based on 100% sequence homology:
Dog

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser536 of human NF-κB p65.

Background

Transcription factors of the nuclear factor κB (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50), and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (3-5). NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression (6-8). NIK and IKKα (IKK1) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which translocates to the nucleus (9-11).

1. Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141-79.
2. Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13-20.
3. Haskill, S. et al. (1991) *Cell* 65, 1281-9.
4. Thompson, J.E. et al. (1995) *Cell* 80, 573-82.
5. Whiteside, S.T. et al. (1997) *EMBO J* 16, 1413-26.
6. Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.
7. Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259-63.
8. Chen, Z.J. et al. (1996) *Cell* 84, 853-62.
9. Senftleben, U. et al. (2001) *Science* 293, 1495-9.
10. Coope, H.J. et al. (2002) *EMBO J* 21, 5375-85.
11. Xiao, G. et al. (2001) *Mol Cell* 7, 401-9.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

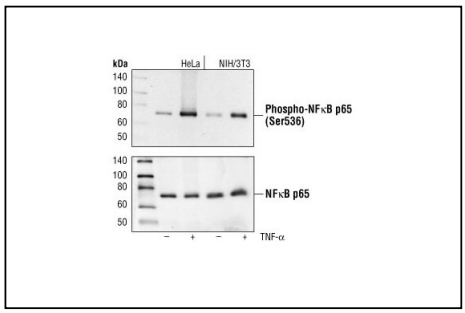
APPLICATIONS KEY **WB:** Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

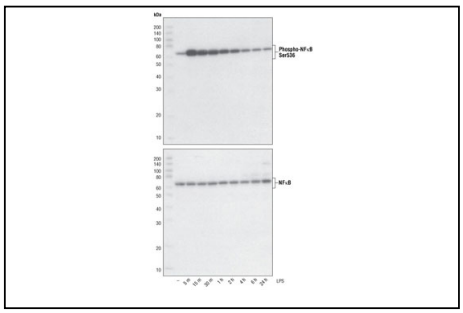
Revision 5

#3033

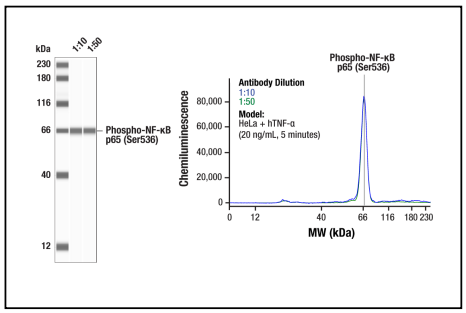
Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb



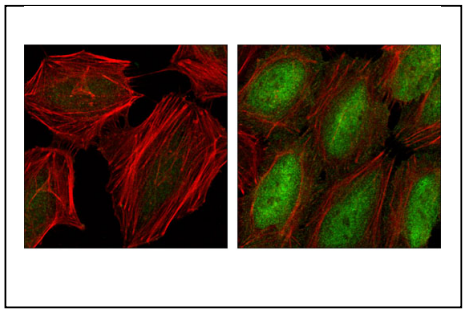
Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or TNF-α treated (#2169, 20 ng/ml for 5 minutes), using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (upper) or NF-κB p65 Antibody #3034 (lower).



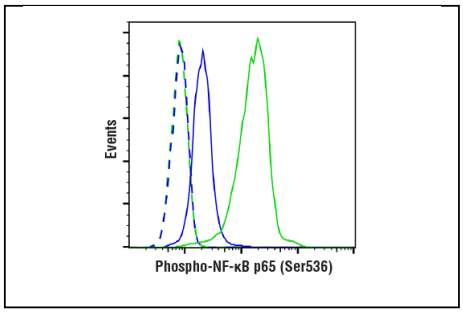
Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24h) and treated with 1 µg/ml LPS for the indicated times, using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (upper) and NF-κB p65 (C22B4) Rabbit mAb #4764 (lower).



Simple Western™ analysis of lysates (1.0 mg/mL) from HeLa cells treated with hTNF-α (20 ng/mL, 5 minutes) using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb #3033. The virtual lane view (left) shows a single target band (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



Confocal immunofluorescent analysis of HeLa cells, serum starved (left) or TNF-α treated (#8902 at 20 ng/ml for 20 min, right), using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® phalloidin 555 (red).



Flow cytometric analysis of HeLa cells, untreated (blue) or treated with Human Tumor Necrosis Factor-α (hTNF-α) #8902 and Calyculin A #9902 (20 ng/ml and 100 nM, 15 min; green), using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

#3033

Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IP, IHC-P, IF-IC, FC-FP, ChIP, ChIP-seq, C&R	H M R Hm Mk Dg	Endogenous	65	Rabbit IgG	Q04206	5970

Product Usage Information

For optimal ChIP and ChIP-seq results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP.

This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400 - 1:1600
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600
Chromatin IP	1:100
Chromatin IP-seq	1:100
CUT&RUN	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #69994.

Specificity / Sensitivity

NF-κB p65 (D14E12) XP® Rabbit mAb recognizes endogenous levels of total NF-κB p65/RelA protein. It does not cross react with other NF-κB/Rel family members.

Species Reactivity:
Human, Mouse, Rat, Hamster, Monkey, Dog

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu498 of human NF-κB p65/RelA protein.

Background

Transcription factors of the nuclear factor κB (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50), and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (3-5). NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression (6-8). NIK and IKKα (IKK1) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which translocates to the nucleus (9-11).

1. Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141-79.
2. Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13-20.
3. Haskill, S. et al. (1991) *Cell* 65, 1281-9.
4. Thompson, J.E. et al. (1995) *Cell* 80, 573-82.
5. Whiteside, S.T. et al. (1997) *EMBO J* 16, 1413-26.
6. Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.
7. Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259-63.
8. Chen, Z.J. et al. (1996) *Cell* 84, 853-62.
9. Senftleben, U. et al. (2001) *Science* 293, 1495-9.
10. Coope, H.J. et al. (2002) *EMBO J* 21, 5375-85.
11. Xiao, G. et al. (2001) *Mol Cell* 7, 401-9.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

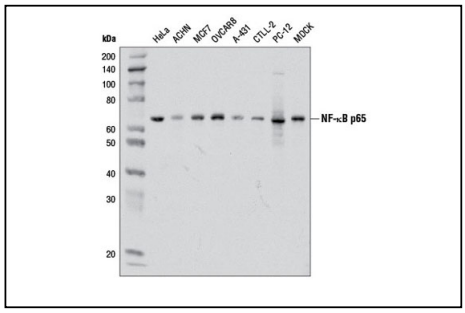
APPLICATIONS KEY **WB:** Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

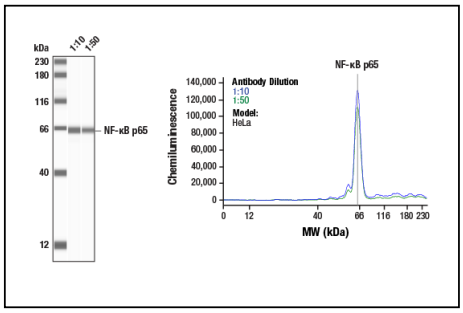
Revision 8

#8242

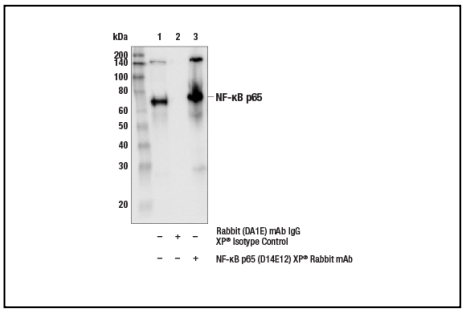
NF-κB p65 (D14E12) XP® Rabbit mAb



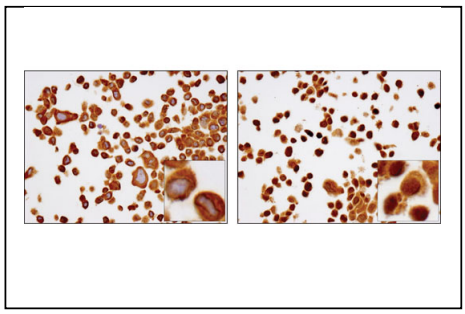
Western blot analysis of extracts from various cell lines using NF-κB p65 (D14E12) XP® Rabbit mAb.



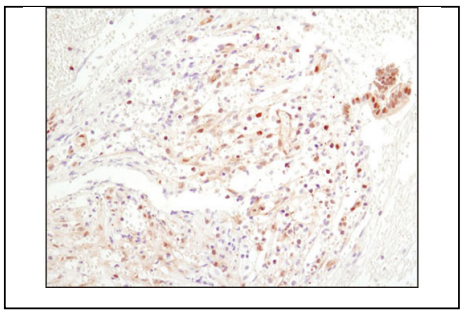
Simple Western™ analysis of lysates (1 mg/mL) from HeLa cells using NF-κB p65 (D14E12) XP® Rabbit mAb #8242. The virtual lane view (left) shows a single target band (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



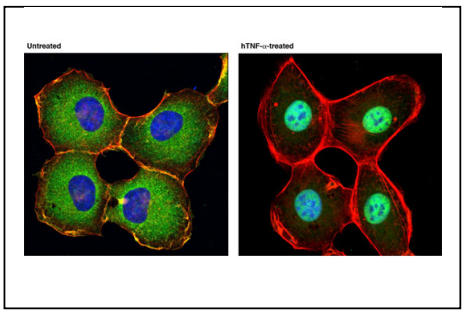
Immunoprecipitation of NF-kB p65 from CHO cell extracts. Lane 1 is 10% input, lane 2 is precipitated with Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is NF-κB p65 (D14E12) XP® Rabbit mAb, #8242. Western blot was performed using NF-κB p65 (L8F6) Mouse mAb, #6956.



Immunohistochemical analysis using NF-κB p65 (D14E12) XP® Rabbit mAb on SignalSlide® NF-κB p65 IHC Controls #12873 (paraffin-embedded HCT116 cells, untreated (left) or treated with hTNF-α #8902 (right)).



Immunohistochemical analysis of paraffin-embedded human chronic cholecystitis using NF-κB p65 (D14E12) XP® Rabbit mAb.

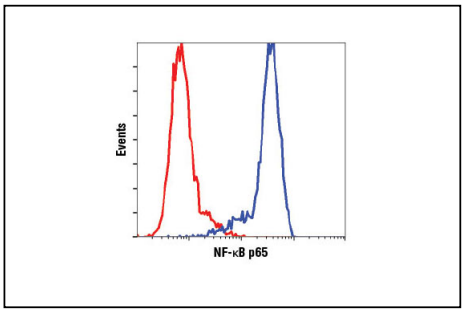


Confocal immunofluorescent analysis of HT-1080 cells, untreated (left) or treated with hTNF-α #8902 (20 ng/ml, 20 min) (right), using NF-κB p65 (D14E12) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

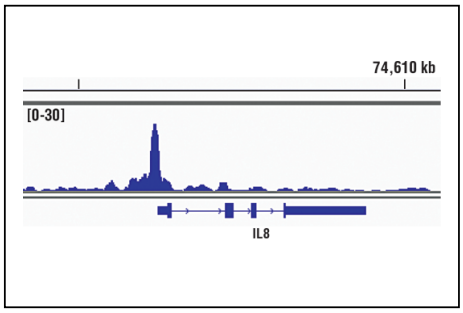
Revision 8

#8242

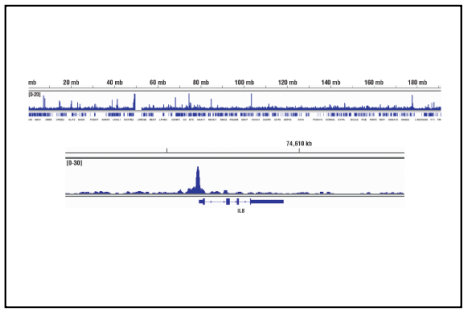
NF-κB p65 (D14E12) XP® Rabbit mAb



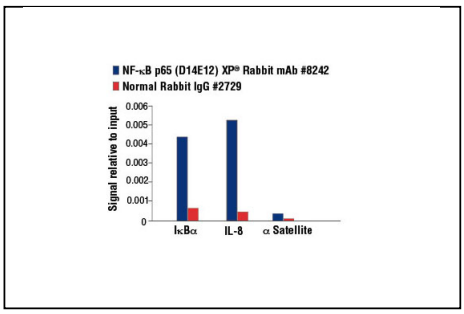
Flow cytometric analysis of HeLa cells using NF-κB p65 (D14E12) XP® Rabbit mAb (blue) compared to concentration matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red).



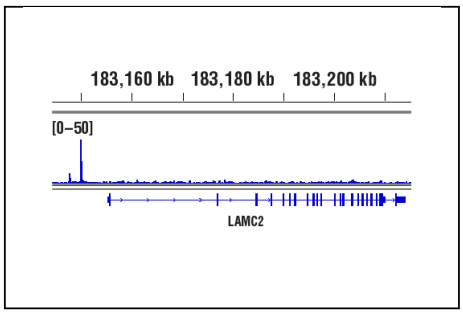
Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and NF-κB p65 (D14E12) XP® Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figure shows binding across IL-8, a known target gene of NFκB (see additional figure containing ChIP-qPCR data). For additional ChIP-seq tracks, please download the product data sheet.



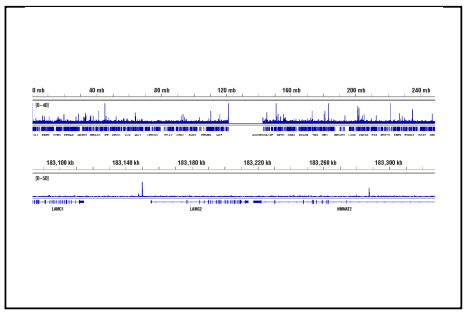
Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and NF-κB p65 (D14E12) XP® Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figure shows binding across chromosome 4 (upper), including IL-8 (lower), a known target gene of NFκB (see additional figure containing ChIP-qPCR data).



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and either NF-κB p65 (D14E12) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using SimpleChIP® Human IκBα Promoter Primers #5552, human IL-8 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.



CUT&RUN was performed with HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and NF-κB p65 (D14E12) XP® Rabbit mAb, using CUT&RUN Assay Kit #86652. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figure shows binding across LAMC2, a known target gene of NF-κB p65 (see additional figure containing CUT&RUN-qPCR data).

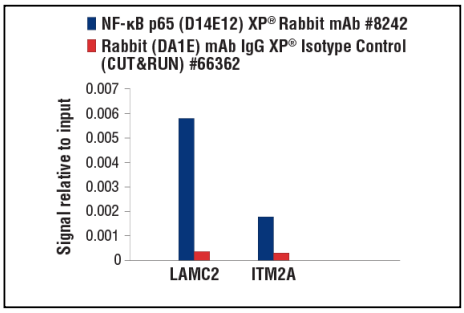


CUT&RUN was performed with HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and NF-κB p65 (D14E12) XP® Rabbit mAb, using CUT&RUN Assay Kit #86652. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figures show binding across chromosome 1 (upper), including LAMC2 (lower), a known target gene of NF-κB p65 (see additional figure containing CUT&RUN-qPCR data).

Revision 8

#8242

NF-κB p65 (D14E12) XP® Rabbit mAb



CUT&RUN was performed with HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and either NF-κB p65 (D14E12) XP® Rabbit mAb or Rabbit (DA1E) mAb IgG XP® Isotype Control (CUT&RUN) #66362, using CUT&RUN Assay Kit #86652. The enriched DNA was quantified by real-time PCR using human LAMC2 upstream primers, and human ITM2A upstream primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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NF-κB p65 (D14E12) XP® Rabbit mAb

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p70 S6 Kinase Antibody

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WB, W-S, IP	H M R Mk	Endogenous	70, 85	Rabbit	P23443	6198

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

p70 S6 Kinase Antibody detects endogenous levels of total p70 S6 kinase protein. This antibody also recognizes p85 S6 kinase.

Species Reactivity:
Human, Mouse, Rat, Monkey

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the carboxy-terminus of human p70 S6 kinase. Antibodies are purified by protein A and peptide affinity chromatography.

Background

p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression (1,2). p70 S6 kinase phosphorylates the S6 protein of the 40S ribosomal subunit and is involved in translational control of 5' oligopyrimidine tract mRNAs (1). A second isoform, p85 S6 kinase, is derived from the same gene and is identical to p70 S6 kinase except for 23 extra residues at the amino terminus, which encode a nuclear localizing signal (1). Both isoforms lie on a mitogen activated signaling pathway downstream of phosphoinositide-3 kinase (PI-3K) and the target of rapamycin, FRAP/mTOR, a pathway distinct from the Ras/MAP kinase cascade (1). The activity of p70 S6 kinase is controlled by multiple phosphorylation events located within the catalytic, linker and pseudosubstrate domains (1). Phosphorylation of Thr229 in the catalytic domain and Thr389 in the linker domain are most critical for kinase function (1). Phosphorylation of Thr389, however, most closely correlates with p70 kinase activity *in vivo* (3). Prior phosphorylation of Thr389 is required for the action of phosphoinositide 3-dependent protein kinase 1 (PDK1) on Thr229 (4,5). Phosphorylation of this site is stimulated by growth factors such as insulin, EGF and FGF, as well as by serum and some G-protein-coupled receptor ligands, and is blocked by wortmannin, LY294002 (PI-3K inhibitor) and rapamycin (FRAP/mTOR inhibitor) (1,6,7). Ser411, Thr421 and Ser424 lie within a Ser-Pro-rich region located in the pseudosubstrate region (1). Phosphorylation at these sites is thought to activate p70 S6 kinase via relief of pseudosubstrate suppression (1,2). Another LY294002 and rapamycin sensitive phosphorylation site, Ser371, is an *in vitro* substrate for mTOR and correlates well with the activity of a partially rapamycin resistant mutant p70 S6 kinase (8).

1. Pullen, N. and Thomas, G. (1997) *FEBS Lett* 410, 78-82.
2. Dufner, A. and Thomas, G. (1999) *Exp Cell Res* 253, 100-9.
3. Weng, Q.P. et al. (1998) *J Biol Chem* 273, 16621-9.
4. Pullen, N. et al. (1998) *Science* 279, 707-10.
5. Alessi, D.R. et al. (1998) *Curr Biol* 8, 69-81.
6. Polakiewicz, R.D. et al. (1998) *J Biol Chem* 273, 23534-41.
7. Fingar, D.C. et al. (2002) *Genes Dev* 16, 1472-87.
8. Saitoh, M. et al. (2002) *J Biol Chem* 277, 20104-12.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

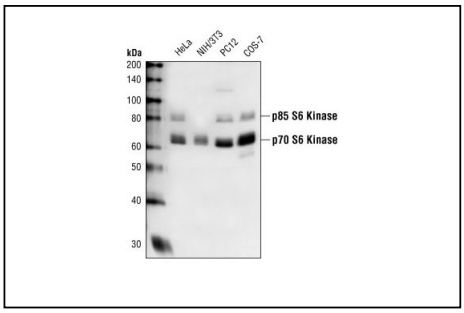
APPLICATIONS KEY **WB:** Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

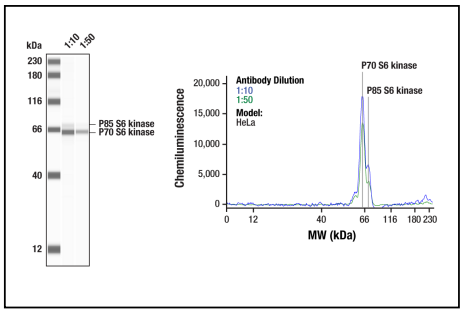
Revision 6

#9202

p70 S6 Kinase Antibody



Western blot analysis of extracts from HeLa, NIH-3T3, PC12 and COS-7 cells using p70 S6 Kinase Antibody.



Simple Western™ analysis of lysates (1 mg/mL) from HeLa cells using p70 S6 Kinase Antibody #9202. The virtual lane view (left) shows a target band for p70 S6 Kinase (as indicated) and p85 S6 Kinase (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.

#9202

p70 S6 Kinase Antibody

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Revision 1

Store at -20C

#9204

Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, IP	H M R Mk	Endogenous	70, 85	Rabbit	P23443	6198

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody detects endogenous levels of p70 S6 kinase only when phosphorylated at Thr421/Ser424. This antibody also detects p85 S6 kinase when phosphorylated at the corresponding sites (Thr444/Ser447).

Species Reactivity:
Human, Mouse, Rat, Monkey

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr421/Ser424 of human p70 S6 kinase. Antibodies are purified by protein A and peptide affinity chromatography.

Background

p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression (1,2). p70 S6 kinase phosphorylates the S6 protein of the 40S ribosomal subunit and is involved in translational control of 5' oligopyrimidine tract mRNAs (1). A second isoform, p85 S6 kinase, is derived from the same gene and is identical to p70 S6 kinase except for 23 extra residues at the amino terminus, which encode a nuclear localizing signal (1). Both isoforms lie on a mitogen activated signaling pathway downstream of phosphoinositide-3 kinase (PI-3K) and the target of rapamycin, FRAP/mTOR, a pathway distinct from the Ras/MAP kinase cascade (1). The activity of p70 S6 kinase is controlled by multiple phosphorylation events located within the catalytic, linker and pseudosubstrate domains (1). Phosphorylation of Thr229 in the catalytic domain and Thr389 in the linker domain are most critical for kinase function (1). Phosphorylation of Thr389, however, most closely correlates with p70 kinase activity *in vivo* (3). Prior phosphorylation of Thr389 is required for the action of phosphoinositide 3-dependent protein kinase 1 (PDK1) on Thr229 (4,5). Phosphorylation of this site is stimulated by growth factors such as insulin, EGF and FGF, as well as by serum and some G-protein-coupled receptor ligands, and is blocked by wortmannin, LY294002 (PI-3K inhibitor) and rapamycin (FRAP/mTOR inhibitor) (1,6,7). Ser411, Thr421 and Ser424 lie within a Ser-Pro-rich region located in the pseudosubstrate region (1). Phosphorylation at these sites is thought to activate p70 S6 kinase via relief of pseudosubstrate suppression (1,2). Another LY294002 and rapamycin sensitive phosphorylation site, Ser371, is an *in vitro* substrate for mTOR and correlates well with the activity of a partially rapamycin resistant mutant p70 S6 kinase (8).

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Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

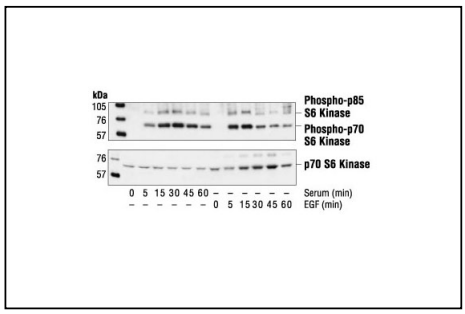
APPLICATIONS KEY **WB:** Western Blotting **IP:** Immunoprecipitation

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

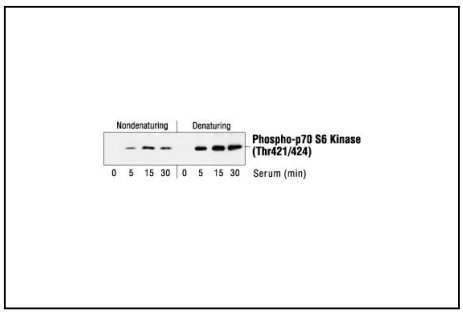
Revision 1

#9204

Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody



Western blot analysis of extracts from 293 cells, untreated, serum-treated or EGF-treated (100 ng/ml), using Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody (upper) or p70 S6 Kinase Antibody #9202 (lower).



Immunoprecipitation of phosphorylated p70 S6 Kinase from 293 cell extracts (cells were serum-stimulated as indicated) under nondenaturing or denaturing conditions, followed by Western blot analysis, using Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody.

#9204

Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody

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Revision 5

Store at -20C

#4283

MyD88 (D80F5) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP	H M R Hm Mk	Endogenous	33	Rabbit IgG	Q99836	4615

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

MyD88 (D80F5) Rabbit mAb detects endogenous levels of total MyD88 protein.

Species Reactivity:
Human, Mouse, Rat, Hamster, Monkey

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Cys233 of human MyD88 protein.

Background

Members of the Toll-like receptor (TLR) family, named for the closely related Toll receptor in *Drosophila*, play a pivotal role in innate immune responses (1-4). TLRs recognize conserved motifs found in various pathogens and mediate defense responses (5-7). Triggering of the TLR pathway leads to the activation of NF-κB and subsequent regulation of immune and inflammatory genes (4). The TLRs and members of the IL-1 receptor family share a conserved stretch of approximately 200 amino acids known as the Toll/Interleukin-1 receptor (TIR) domain (1). Upon activation, TLRs associate with a number of cytoplasmic adaptor proteins containing TIR domains, including myeloid differentiation factor 88 (MyD88), MyD88-adaptor-like/TIR-associated protein (MAL/TIRAP), Toll-receptor-associated activator of interferon (TRIF), and Toll-receptor-associated molecule (TRAM) (8-10). This association leads to the recruitment and activation of IRAK1 and IRAK4, which form a complex with TRAF6 to activate TAK1 and IKK (8,11-14). Activation of IKK leads to the degradation of IκB, which normally maintains NF-κB in an inactive state by sequestering it in the cytoplasm.

MyD88 was originally isolated as a myeloid differentiation primary response gene that is rapidly induced upon IL-6 stimulated differentiation of M1 myeloleukemic cells into macrophages (15-17). It contains an amino-terminal death domain separated from a carboxyl-terminal TIR domain and functions as an adaptor in TLR/IL-1 receptor signaling (18). The death domain of MyD88 mediates interactions with the IRAK complex triggering a signaling cascade that includes the activation of NF-κB (19,20).

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20. Muzio, M. et al. (1997) *Science* 278, 1612-5.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

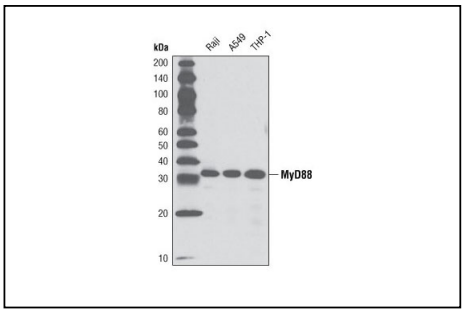
APPLICATIONS KEY **WB:** Western Blotting **IP:** Immunoprecipitation

CROSS-REACTIVITY KEY **H:** human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

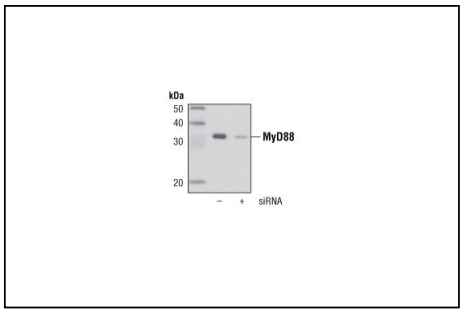
Revision 5

#4283

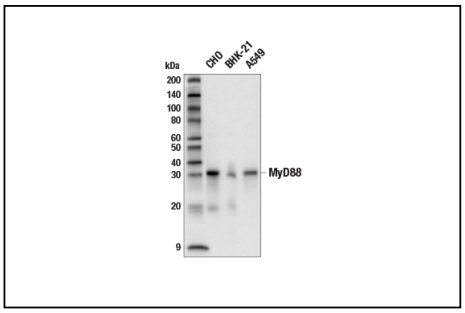
MyD88 (D80F5) Rabbit mAb



Western blot analysis of extracts from Raji, A549, and THP-1 cells using MyD88 (D80F5) Rabbit mAb.



Western blot analysis of extracts from A549 cells, untransfected () or transfected with a MyD88 siRNA (+), using MyD88 (D80F5) Rabbit mAb.



Western blot analysis of extracts from CHO, BHK-21, and A549 cells using MyD88 (D80F5) Rabbit mAb.

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MyD88 (D80F5) Rabbit mAb

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Ultra-LEAF™ Purified anti-human CD3 Antibody

Catalog# / Size	317325 / 100 µg 317326 / 1 mg 317347 / 5 mg 317348 / 25 mg 317349 / 50 mg 317350 / 100 mg
Clone	OKT3
Regulatory Status	RUO
Workshop	HCDM listed
Other Names	T3, CD3ε
Isotype	Mouse IgG2a, κ
Description	CD3ε is a 20 kD chain of the CD3/T cell receptor (TCR) complex, which is composed of two CD3ε, one CD3γ, one CD3δ, one CD3ζ (CD247), and a T cell receptor (α/β or γ/δ) heterodimer. It is found on all mature T lymphocytes, NK T cells, and some thymocytes. CD3, also known as T3, is a member of the immunoglobulin superfamily that plays a role in antigen recognition, signal transduction, and T cell activation.

Product Details

Verified Reactivity	Human
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	0.2 µm filtered in phosphate-buffered solution, pH 7.2, containing no preservative. Endotoxin level is <0.01 EU/µg of the protein (<0.001 ng/µg of the protein) as determined by the LAL test.
Preparation	The Ultra-LEAF™ (Low Endotoxin, Azide-Free) antibody was purified by affinity chromatography.
Concentration	The antibody is bottled at the concentration indicated on the vial, typically between 2 mg/mL and 3 mg/mL. Older lots may have also been bottled at 1 mg/mL. To obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C. This Ultra-LEAF™ solution contains no preservative; handle under aseptic conditions.
Application	FC - *Quality tested*
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 2.0 µg per million cells in 100 µl volume or 100 µl of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	The OKT3 monoclonal antibody reacts with an epitope on the epsilon-subunit within the human CD3 complex. Clone OKT3 can block the binding of clones SK7 and UCHT1.[4] The OKT3 antibody is able to induce T cell activation. Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen sections and activation of T cells. The LEAF™ purified antibody (Endotoxin <0.1 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for functional assays (Cat. No. 317304). For highly sensitive assays, we recommend Ultra-LEAF™ purified antibody (Cat. No. 317326) with a lower endotoxin limit than standard LEAF™ purified antibodies (Endotoxin <0.01 EU/µg).
Application References **(PubMed link indicates BioLegend citation)**	1. Schlossman S, *et al.* Eds. 1995. Leucocyte Typing V. Oxford University Press. New York. 2. Knapp W. 1989. Leucocyte Typing IV. Oxford University Press New York. 3. Barclay N, *et al.* 1997. The Leucocyte Antigen Facts Book. Academic Press Inc. San Diego.

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AB_11150592 (BioLegend Cat. No. 317326)
AB_2571994 (BioLegend Cat. No. 317347)
AB_2571995 (BioLegend Cat. No. 317348)
AB_2749888 (BioLegend Cat. No. 317349)
AB_2749889 (BioLegend Cat. No. 317350)

Antigen Details

Structure	Ig superfamily, the subunits CD3γ, CD3δ, CD3ζ (CD247) and TCR (α/β or γ/δ) form the CD3/TCR complex, 20 kD
Distribution	Mature T and NK T cells, thymocyte differentiation
Function	Antigen recognition, signal transduction, T cell activation
Ligand/Receptor	Peptide antigen bound to MHC
Cell Type	NKT cells, T cells, Thymocytes, Tregs
Biology Area	Immunology
Molecular Family	CD Molecules
Antigen References	1. Barclay N, *et al.* 1993. The Leucocyte FactsBook. Academic Press. San Diego. 2. Beverly P, *et al.* 1981. *Eur. J. Immunol.* 11:329. 3. Lanier L, *et al.* 1986. *J. Immunol.* 137:2501.
Gene ID	916

Related Protocols

Cell Surface Flow Cytometry Staining Protocol

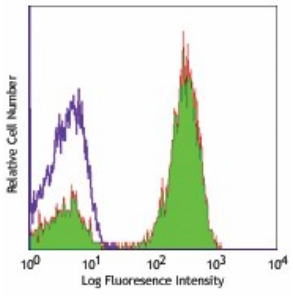
T Cell Activation with anti-CD3 Antibodies Protocol - Human

T Cell Activation with anti-CD3 Antibodies Protocol - Mouse

Other Formats

Purified anti-human CD3, FITC anti-human CD3, PE anti-human CD3, Alexa Fluor® 488 anti-human CD3, Alexa Fluor® 647 anti-human CD3, Pacific Blue™ anti-human CD3, APC anti-human CD3, Biotin anti-human CD3, Brilliant Violet 605™ anti-human CD3, Brilliant Violet 650™ anti-human CD3, Ultra-LEAF™ Purified anti-human CD3, Brilliant Violet 711™ anti-human CD3, Brilliant Violet 785™ anti-human CD3, Brilliant Violet 510™ anti-human CD3, PE/Cyanine7 anti-human CD3, PerCP/Cyanine5.5 anti-human CD3, PerCP anti-human CD3, Alexa Fluor® 700 anti-human CD3, APC/Cyanine7 anti-human CD3, Brilliant Violet 421™ anti-human CD3, PE/Dazzle™ 594 anti-human CD3, APC/Fire™ 750 anti-human CD3, GMP Ultra-LEAF™ Purified anti-human CD3 SF, PE/Cyanine5 anti-human CD3 Antibody

Product Data



Human peripheral blood lymphocytes stained with LEAF™ purified OKT3, followed by anti-mouse IgG FITC

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Ultra-LEAF™ Purified anti-human CD28 Antibody

Catalog# / Size	302933 / 100 µg 302934 / 1 mg 302943 / 5 mg 302944 / 25 mg 302959 / 50 mg 302960 / 100 mg
Clone	CD28.2
Regulatory Status	RUO
Workshop	V-CD28.05
Other Names	T44, Tp44
Isotype	Mouse IgG1, κ
Description	CD28 is a 44 kD disulfide-linked homodimeric type I glycoprotein. It is a member of the immunoglobulin superfamily and is also known as T44 or Tp44. CD28 is expressed on most T lineage cells, NK cell subsets, and plasma cells. CD28 binds both CD80 and CD86 using a highly conserved motif MYPPY in the CDR3-like loop. CD28 is considered a major co-stimulatory molecule, inducing T lymphocyte activation and IL-2 synthesis, and preventing cell death. *In vitro* studies indicate that ligation of CD28 on T cells by CD80 and CD86 on antigen presenting cells provides a costimulatory signal required for T cell activation and proliferation.

Product Details

Verified Reactivity	Human, Cynomolgus, Rhesus
Reported Reactivity	Baboon, Capuchin Monkey, Chimpanzee, Pigtailed Macaque, Sooty Mangabey, Squirrel Monkey
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	0.2 µm filtered in phosphate-buffered solution, pH 7.2, containing no preservative. Endotoxin level is <0.01 EU/µg of the protein (<0.001 ng/µg of the protein) as determined by the LAL test.
Preparation	The Ultra-LEAF™ (Low Endotoxin, Azide-Free) antibody was purified by affinity chromatography.
Concentration	The antibody is bottled at the concentration indicated on the vial, typically between 2 mg/mL and 3 mg/mL. Older lots may have also been bottled at 1 mg/mL. To obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C. This Ultra-LEAF™ solution contains no preservative; handle under aseptic conditions.
Application	FC - *Quality tested* IHC-F, Costim, FA - *Reported in the literature, not verified in house*
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 1.0 µg per million cells in 100 µl volume or 100 µl of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	The Ultra-LEAF™ Purified antibody (Endotoxin < 0.01 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for highly sensitive assays.
Application References **(PubMed link indicates BioLegend citation)**	1. Schlossman S, *et al.* Eds. 1995. Leucocyte Typing V. Oxford University Press. New York. 2. Nunes J, *et al.* 1993. *Biochem. J.* 293:835. 3. Calea-Lauri J, *et al.* 1999. *J. Immunol.* 163:62. 4. Tazi A, *et al.* 1999. *J. Immunol.* 163:3511. (IHC) 5. Marti F, *et al.* 2001. *J. Immunol.* 166:197. (Costim) 6. Jeong SH, *et al.* 2004. *J. Virol.* 78:6995. (Costim)

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RRID

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AB_11148949 (BioLegend Cat. No. 302934)
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AB_2800748 (BioLegend Cat. No. 302959)
AB_2800749 (BioLegend Cat. No. 302960)

Antigen Details

Structure	Ig superfamily, type I transmembrane glycoprotein, homodimer, 44 kD
Distribution	Mature T cells, thymocytes, NK cell subsets, plasma cells, EBV-positive B cells
Function	T cell costimulation
Ligand/Receptor	CD80, CD86
Cell Type	B cells, NK cells, Plasma cells, T cells, Thymocytes, Tregs
Biology Area	Costimulatory Molecules, Immunology
Molecular Family	CD Molecules
Antigen References	1. Schlossman S, *et al.* Eds. 1995. Leucocyte Typing V. Oxford University Press. New York. 2. June CH, *et al.* 1994. *Immunol. Today* 15:321. 3. Linskey PS, *et al.* 1993. *Annu. Rev. Immunol.* 11:191.
Gene ID	940

Related Protocols

Cell Surface Flow Cytometry Staining Protocol

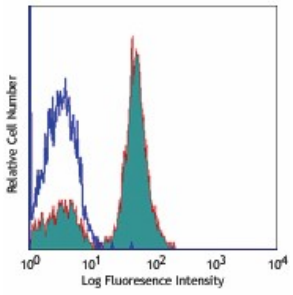
T Cell Activation with anti-CD3 Antibodies Protocol - Human

T Cell Activation with anti-CD3 Antibodies Protocol - Mouse

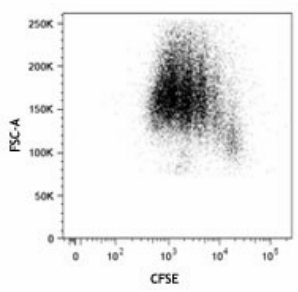
Other Formats

APC anti-human CD28, Biotin anti-human CD28, FITC anti-human CD28, PE anti-human CD28, PE/Cyanine5 anti-human CD28, Purified anti-human CD28, Alexa Fluor® 488 anti-human CD28, Alexa Fluor® 700 anti-human CD28, PerCP/Cyanine5.5 anti-human CD28, Pacific Blue™ anti-human CD28, PE/Cyanine7 anti-human CD28, Ultra-LEAF™ Purified anti-human CD28, Brilliant Violet 421™ anti-human CD28, Brilliant Violet 510™ anti-human CD28, Purified anti-human CD28 (Maxpar® Ready), PE/Dazzle™ 594 anti-human CD28, Brilliant Violet 785™ anti-human CD28, Brilliant Violet 650™ anti-human CD28, Brilliant Violet 711™ anti-human CD28, APC/Fire™ 750 anti-human CD28, Alexa Fluor® 647 anti-human CD28, TotalSeq™-A0386 anti-human CD28, TotalSeq™-B0386 anti-human CD28, TotalSeq™-C0386 anti-human CD28, Brilliant Violet 605™ anti-human CD28, APC/Cyanine7 anti-human CD28, Brilliant Violet 750™ anti-human CD28, PE/Fire™ 810 anti-human CD28, GMP PE anti-human CD28, TotalSeq™-D0386 anti-human CD28, Spark Violet™ 423 anti-human CD28

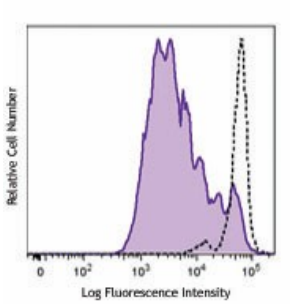
Product Data



Human peripheral blood lymphocytes stained with LEAF™ purified CD28.2, followed by anti-mouse IgGs FITC



Human peripheral blood mononuclear cells were stained with CFSE on day 0, and then stimulated with (filled histogram) or without (open histogram) immobilized LEAF™ Purified CD3 (clone UCHT1) and LEAF™ purified CD28 (clone CD28.2) for 3 days. On day 4, cells were harvested and stained with CD4 Brilliant Violet 711™. Dot plot (above) was analyzed on live cells. Histogram data (below) was analyzed by gating on CD4 positive cells (above).



Human peripheral blood mononuclear cells were stained with CFSE on day 0, and then stimulated with (filled histogram) or without (open histogram) immobilized LEAF™ Purified CD3 (clone UCHT1) and LEAF™ purified CD28 (clone CD28.2) for 3 days. On day 4, cells were harvested and stained with CD4 Brilliant Violet 711™. Dot plot (above) was analyzed on live cells. Histogram data (below) was analyzed by gating on CD4 positive cells (above).

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