Version: 0 Revision Date: 05.11.2017



## LEGENDplex™ Human MMP-9 Capture Bead B7, 13X

Catalog# / Size 740565 / 100 tests (270 μL)

Regulatory Status RUO

Other Names MMP9, Matrix Metallopeptidase 9, Matrix Metalloproteinase 9, Gelatinase B, 92kDa Gelatinase,

92kDa Type IV Collagenase, Matrix Metalloproteinase-9, EC 3.4.24.35, CLG4B, GELB

Description The Vascular Inflammation Panel 1 Mix and Match Capture Beads offer flexible customization

within the panel (13-plex) with the following items listed below:
740555 LEGENDplex™ Human Myoglobin Capture Bead A4, 13X
740556 LEGENDplex™ Human MRP8/14 Capture Bead A5, 13X
740557 LEGENDplex™ Human NGAL Capture Bead A6, 13X
740558 LEGENDplex™ Human CRP Capture Bead A7, 13X
740559 LEGENDplex™ Human MMP-2 Capture Bead A10, 13X
740560 LEGENDplex™ Human OPN Capture Bead A10, 13X
740561 LEGENDplex™ Human MPO Capture Bead B2, 13X
740562 LEGENDplex™ Human IGFBP-4 Capture Bead B4, 13X
740563 LEGENDplex™ Human ICAM-1 Capture Bead B5, 13X
740564 LEGENDplex™ Human VCAM-1 Capture Bead B6, 13X
740565 LEGENDplex™ Human MMP-9 Capture Bead B7, 13X
740566 LEGENDplex™ Human MMP-9 Capture Bead B7, 13X

Selected items will be used together with:

740554 LEGENDplex™ Human Vascular Inflammation Panel 1 Standard

740553 LEGENDplex™ Human Vascular Inflammation Panel 1 Detection Antibodies

740374 LEGENDplex™ Buffer Set C

740377 or 740379 Filter Plate or V-bottom Plate

The assembled Human Vascular Inflammation Mix and Match Panel 1 is a multiplex bead-based assay kit, using fluorescence—encoded beads suitable for use on various flow cytometers. This allows simultaneous quantification of up to 13 human proteins: Myoglobin, MRP8/14, NGAL, CRP, MMP-2, OPN, MPO, SAA, IGFBP-4, ICAM-1, VCAM-1, MMP-9, and Cystatin C. This assay panel provides higher detection sensitivities and broader dynamic ranges than traditional ELISA methods. The panel has been validated for use with serum, plasma, tissue culture supernatant, and urine samples.

#### **Product Details**

Verified Reactivity Human

Application <u>Multiplex</u>

Learn more about LEGENDplex™ at biolegend.com/legendplex

Download the  $\underline{\mathsf{LEGENDplex}^\mathsf{TM}}$  software here.

#### Antigen Details

Biology Area Angiogenesis, Cell Adhesion, Cell Biology, Neuroinflammation, Neuroscience

Molecular Family Enzymes and Regulators

Gene ID 4318

#### **Related Protocols**

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## LEGENDplex™ Human MMP-2 Capture Bead A8, 13X

Catalog# / Size 740559 / 100 tests (270 μL)

Regulatory Status RUO

Other Names Matrix Metallopeptidase 2, Matrix Metalloproteinase-2, EC 3.4.24.24, CLG4A, TBE-1, Matrix

Metalloproteinase 2, Gelatinase A, 72kDa Gelatinase, 72kDa Type IV Collagenase, Matrix

Metalloproteinase-II

**Description** The Vascular Inflammation Panel 1 Mix and Match Capture Beads offer flexible customization

within the panel (13-plex) with the following items listed below: 740555 LEGENDplex™ Human Myoglobin Capture Bead A4, 13X 740556 LEGENDplex™ Human MRP8/14 Capture Bead A5, 13X 740557 LEGENDplex™ Human NGAL Capture Bead A6, 13X 740558 LEGENDplex™ Human CRP Capture Bead A7, 13X 740559 LEGENDplex™ Human MMP-2 Capture Bead A8, 13X 740560 LEGENDplex™ Human OPN Capture Bead A10, 13X 740561 LEGENDplex™ Human MPC Capture Bead B2, 13X 740562 LEGENDplex™ Human SAA Capture Bead B3, 13X 740476 LEGENDplex™ Human IGFBP-4 Capture Bead B4, 13X 740563 LEGENDplex™ Human ICAM-1 Capture Bead B5, 13X 740564 LEGENDplex™ Human VCAM-1 Capture Bead B6, 13X 740565 LEGENDplex™ Human VCAM-1 Capture Bead B7, 13X 740566 LEGENDplex™ Human MMP-9 Capture Bead B7, 13X 740566 LEGENDplex™ Human Cystatin C Capture Bead B9, 13X

Selected items will be used together with:

740554 LEGENDplex™ Human Vascular Inflammation Panel 1 Standard

740553 LEGENDplex™ Human Vascular Inflammation Panel 1 Detection Antibodies

740374 LEGENDplex™ Buffer Set C

740377 or 740379 Filter Plate or V-bottom Plate

The assembled Human Vascular Inflammation Mix and Match Panel 1 is a multiplex bead-based assay kit, using fluorescence—encoded beads suitable for use on various flow cytometers. This allows simultaneous quantification of up to 13 human proteins: Myoglobin, MRP8/14, NGAL, CRP, MMP-2, OPN, MPO, SAA, IGFBP-4, ICAM-1, VCAM-1, MMP-9, and Cystatin C. This assay panel provides higher detection sensitivities and broader dynamic ranges than traditional ELISA methods. The panel has been validated for use with serum, plasma, tissue culture supernatant, and urine samples.

#### **Product Details**

Verified Reactivity Human

Application Multiplex

Learn more about LEGENDplex™ at biolegend.com/legendplex

Download the <u>LEGENDplex™ software here</u>.

**Antigen Details** 

Biology Area Angiogenesis, Cell Adhesion, Cell Biology, Neuroinflammation, Neuroscience

Molecular Family Enzymes and Regulators

Gene ID <u>4313</u>

**Related Protocols** 

LEGENDplex™ Laboratory Protocol - Video

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## Purified anti-MMP-9 Antibody (Previously Covance catalog# MMS-5163)

Catalog# / Size 819701 / 100 μL

Clone L51/82
Regulatory Status RUO

Other Names GELB, MMP9, gelatinase B, 92 kD gelatinase, 92 kD type IV collagenase, 92-kD type IV

collagenase, matrix metalloproteinase 9 (gelatinase B 92-kD type IV collagenase), matrix

metalloproteinase 9 (gelatinase B, 92-kD type IV collagenase)

Previously Covance Catalog# MMS-5163

**Isotype** Mouse IgG2a

**Description** Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade

substances within the extracellular matrix. The MMP family includes different groups of enzymes, such as, collagenases, gelatinases, stromelysins, transmembrane MMPs, matrilysins and others. MMPs are secreted as proenzymes that have to be cleaved in order to be activated. Other MMPs, plasmins as well as other factors activate MMPs. MMPs are thought to play an important role in tissue remodeling associated with various physiological and pathological processes. MMP9 degrades proteins in the extracellular matrix and activates growth factors like proTGFb and proTNFa. MMP-9 contributes to the invasion and metastasis of various human malignancies. Clone F11P2C3 has been shown to be useful for western

blotting and immunohistochemistry of human MMP9.

#### **Product Details**

Verified Reactivity Human, Mouse, Rat

Antibody Type Monoclonal

Host Species Mouse

Immunogen This monoclonal antibody was raised against a full-length fusion protein corresponding to amino

acids 1-708 of rat MMP9 protein.

**Formulation** Phosphate-buffered solution.

**Preparation** The antibody was purified by affinity chromatography.

Concentration 1 mg/ml

Storage & Handling The antibody solution should be stored undiluted between 2°C and 8°C. Please note the storage

condition for this antibody has been changed from -20°C to between 2°C and 8°C. You can also check your vial or your CoA to find the most accurate storage condition for this antibody.

Application <u>IHC-P - Quality tested</u>

WB - Verified

Recommended Usage Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded

immunohistochemical staining. For immunohistochemistry, a concentration range of  $1.0 - 10.0 \, \mu \text{g/mL}$  is suggested. For western blotting, the suggested use of this reagent is  $1.0 - 10.0 \, \mu \text{g/mL}$ . It is recommended that the reagent be titrated for optimal performance for each application.

**Product Citations** 

1. Kuo PC, et al. 2021. Brain Commun. 3:fcab187. PubMed

2. Lee JH, et al. 2022. EBioMedicine. 77:103903. PubMed

3. Ito H, et al. 2021. Neuropsychopharmacology. 46:442. PubMed

4. Swieboda D, et al. 2020. Front Immunol. 1.697916667. PubMed

5. Brezovakova V, et al. 2022. Cells. 11:. PubMed

**RRID** AB 2564833 (BioLegend Cat. No. 819701)

#### **Antigen Details**

Structure Expected MW: 82 and 92 kD

Biology Area Angiogenesis, Cell Adhesion, Cell Biology, Neuroinflammation, Neuroscience

Molecular Family Enzymes and Regulators

Gene ID <u>81687</u>

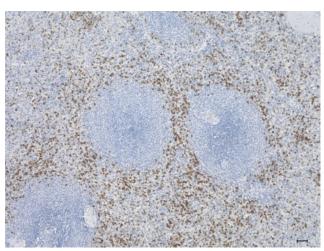
#### **Related Protocols**

• Immunohistochemistry Protocol for Paraffin-Embedded Sections

#### **Other Formats**

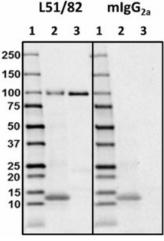
Purified anti-MMP-9

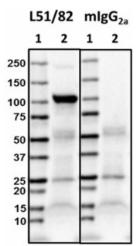
## **Product Data**



IHC staining of purified anti-MMP-9 antibody (clone L51/82) on formalin-fixed paraffin-embedded human spleen tissue. Following antigen retrieval using Sodium Citrate H.I.E.R., the tissue was incubated with 10  $\mu$ g/mL of the primary antibody overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP Detection Kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. The image was captured with a 10X objective. Scale bar: 50  $\mu$ m

IHC staining of purified anti-MMP-9 antibody (clone L51/82) on formalin-fixed paraffin-embedded human spleen tissue. Following antigen retrieval using Sodium Citrate H.I.E.R., the tissue was incubated with 10 µg/mL of the primary antibody overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP Detection Kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. The image was captured with a 40X objective. Scale bar: 50 µm





Western blot of purified anti-MMP-9 antibody (clone L51/82). Lanes 1: Molecular weight marker; Lanes 2:20 µg of human spleen lysate; Lanes 3:20 µg of rat spleen lysate. The blots were incubated with 5 µg/mL of clone L51/82 or mouse lgG2a overnight at 4°C, followed by incubation with HRP goat anti-mouse lgG antibody (Cat. No. 405306). Enhanced chemiluminescence was used as the detection system.

Western blot of purified anti-MMP-9 antibody (clone L51/82). Lanes 1: Molecular weight marker; Lanes 2:20 µg of mouse spleen lysate. The blots were incubated with 1 µg/mL of clone L51/82 or mouse IgG2a overnight at 4°C, followed by incubation with HRP goat anti-mouse IgG antibody (Cat. No. 405306). Enhanced chemiluminescence was used as the detection system.

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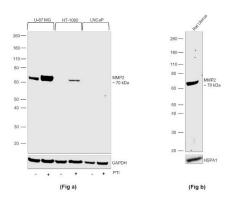


# MMP2 Monoclonal Antibody (101)

<b>Product Details</b>	
Size	100 μg
Species Reactivity	Human, Mouse, Non-human primate, Rabbit, Rhesus monkey, Rat
Published Species	Fish, Human, Mouse
Host/Isotype	Mouse / IgG1
Class	Monoclonal
Туре	Antibody
Clone	101
Conjugate	Unconjugated
Immunogen	Recombinant protein derived from the N-terminus of human MMP-2 protein (accession # P08253, NP_004521), which is identical to Rhesus monkey and chimpanzee and 98% similar to rat and rabbit and 97% to mouse.
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS, pH 7.4
Contains	0.1% sodium azide
Storage conditions	-20°C
RRID	AB_2532214

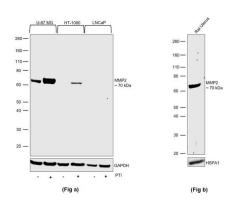
Applications	Tested Dilution	Publications
Western Blot (WB)	2 μg/mL	7 Publications
Immunohistochemistry (IHC)	-	3 Publications
Immunohistochemistry (Paraffin) (IHC (P))	1:20-1:200	-
Immunohistochemistry (Frozen) (IHC (F))	-	1 Publication
Immunocytochemistry (ICC/IF)	2 μg/mL	1 Publication
Flow Cytometry (Flow)	3-5 µg/1x10^6 cells	1 Publication
Immunoprecipitation (IP)	Assay-dependent	-

**Product Images For MMP2 Monoclonal Antibody (101)** 



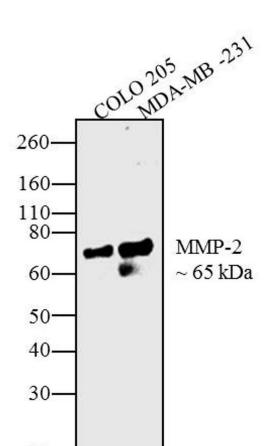
#### MMP2 Antibody (436000)

Antibody specificity was demonstrated by detection of differential basal expression of the target across the cell lines tested owing to their inherent genetic constitution. In Fig a, Relative expression of MMP2 was observed in U-87 MG treated with PTI and HT-1080 treated with PTI in comparison with LNCaP treated with PTI, a cell line that expresses very low levels of MMP2 (https://doi.org/10.1210/en.2002-0157), using Anti-MMP2 Monoclonal Antibody (101) (Product # 436000) in Western Blot. {RE}



#### MMP2 Antibody (436000) in WB

Western blot was performed using Anti-MMP2 Monoclonal Antibody (101) (Product # 436000) and a 70 kDa band corresponding to MMP2 was observed. For Fig a, Whole cell extracts (40 µg lysate) of U-87 MG (Lane 1), U-87 MG treated with Protein Transport Inhibitor (PTI) cocktail (1X, 4 hours) (Lane 2), HT-1080 (Lane 3), HT-1080 treated with Protein Transport Inhibitor (PTI) cocktail (1X, 4 hours) (Lane 4), LNCaP (Lane 5), LNCaP treated with Protein Transport Inhibitor (PTI) cocktail (1X, 4 hours) (Lane 6) and for Fig b, tissue extract of Rat Uterus were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (2 μg/mL) and detected by chemiluminescence with Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177,1: 4000) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005), Relative expression was observed in U-87 MG treated with PTI and HT-180 treated with PTI in comparison with LNCaP treated with PTI.



#### MMP2 Antibody (436000) in WB

Western blot analysis was performed on conditioned media of Serum Starved COLO 205 (Lane 1) and Serum Starved MDA-MB-231 (Lane 2). The blots were probed with Anti-MMP-2 Mouse Monoclonal Antibody (Product # 436000, 2 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.4 µg/mL, 1:2500 dilution). A ~ 65 kDa band corresponding to MMP-2 was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 10 % Bis-Tris gel (Product # NP0302BOX), XCell SureLock™ Electrophoresis System (Product # El0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).

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## View more figures on thermofisher.com

#### □ 13 References

## Western Blot (7)

#### Cancers

## Ciliary Neurotrophic Factor Modulates Multiple Downstream Signaling Pathways in Prostate Cancer Inhibiting Cell Invasiveness.

"436000 was used in Western Blotting to show that CNTF is expressed in PCa and CRPC tissues, and has a pivotal role in prostate cancer environment remodeling and as a negative modulator of invasion processes of CRPC cell models.

Authors: Tossetta G,Fantone S,Gesuita R,Goteri G,Senzacqua M,Marcheggiani F,Tiano L,Marzioni D,Mazzucchelli R

Year 2022

**Species** Human

Dilution 1:500

#### Pharmacology

## EIF5A2 Is Involved in the Biological Process of Cervical Cancer Cells through AGR2.

"436000 was used in Western Blotting to show that EIF5A2 knockdown inhibited the biological process of cervical cancer cells through modulation of AGR2.

Authors: Shen X.Li L.He Y.Lv X.Ma J

Year 2022

**Species** Human

Dilution 1:600

View more WB references on thermofisher.com

## Immunohistochemistry (3)

#### Journal of ovarian research

## Expression of metalloproteinases MMP-2 and MMP-9 is associated to the presence of androgen receptor in epithelial ovarian tumors.

"436000 was used in Immunohistochemistry to show that MMP-2 located in the epithelium and the stroma are independent prognostic biomarkers for overall survival in epithelial ovarian tumors.'

Authors: Morales-Vásquez F,Castillo-Sánchez R,Gómora MJ,Almaraz MÁ,Pedernera E,Pérez-Montiel D,Rendón E, López-Basave HN, Román-Basaure E, Cuevas-Covarrubias S, Maldonado-Cubas J, Villa A, Mendez C

Year 2020

**Species** Human

Proceedings of the National Academy of Sciences of the United States of America

## A hypoxia-induced Rab pathway regulates embryo implantation by controlled trafficking of secretory granules.

"436000 was used in Immunohistochemistry to provide an insight into the intracellular communication mechanisms that operate during adaptation to hypoxia, which is essential for embryo implantation and establishment of pregnancy."

Authors: Bhurke A,Kannan A,Neff A,Ma Q,Laws MJ,Taylor RN,Bagchi MK,Bagchi IC

Year 2020

**Species** Mouse

Dilution 1:200

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## More applications with references on thermofisher.com

IHC (F) (1) ICC/IF (1) Flow (1)

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## **Product Information**

**Catalog Number:** 115-605-003

Product: Alexa Fluor® 647-AffiniPure Goat Anti-Mouse IgG (H+L)

Physical State: Freeze-dried solid

**Size:** 1.5 mg

**Antibody Concentration:** ± 1.5 mg/ml (exact concentration lot dependent)

Fluorophore: Alexa Fluor®647 carboxylic acid

Amax = 651 nm; Emax = 667 nm

Suggested Dilution Range: 1:100 - 1:800 for most applications

**Buffer:** 0.01M Sodium Phosphate, 0.25M NaCl, pH 7.6

Stabilizer: 15 mg/ml Bovine Serum Albumin (lgG-Free, Protease-Free)

**Preservative:** 0.05% Sodium Azide

**Purity:** The antibody was purified from antisera by immunoaffinity chromatography using antigens

coupled to agarose beads.

Antibody Specificity: Based on immunoelectrophoresis and/or ELISA, the antibody reacts with whole molecule

mouse IgG. It also reacts with the light chains of other mouse immunoglobulins. No antibody was detected against non-immunoglobulin serum proteins. The antibody may cross-react with

immunoglobulins from other species.

Reconstitution and Storage: Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with 1.5 ml dH2O (exact

volume is lot specific) and centrifuge if not clear. Product is stable for about 6 weeks at 2-8°C as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, aliquot and freeze at -70°C or below. Avoid repeated freezing and thawing. Alternatively, add an equal volume of glycerol (ACS grade or better) for a final concentration of 50%, and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein

concentration and dilution range by one-half.

Expiration date:one year from date of rehydration. The expiration date may be extended if test

results are acceptable for the intended use.

**Safety Information:** Standard Laboratory Practices should be followed when handling this material. The chemical,

physical, and toxicological properties of this material have not been thoroughly investigated. The antibody contains 0.05% sodium azide (a poisonous and hazardous substance) as preservative. Although this concentration is not regarded as dangerous to health, appropriate

measures should be taken to avoid skin and eye contact, inhalation, and ingestion.

**Note:** This information sheet contains general product information only. For detailed lot specific information consult the vial label and the data sheet supplied by the manufacturer upon delivery of the product.

This product is for In Vitro experimental use only. Not for Therapeutic or Diagnostic use.

Manufacturer:

Jackson ImmunoResearch Laboratories, Inc. 872 West Baltimore Pike West Grove, PA, USA 19390

**Distribution and Support:** 

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Fax: 040 45 06 74 90
Email: order@dianova.de





## Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555

<b>Product Details</b>	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Туре	Secondary Antibody
Conjugate	Alexa Fluor™ 555
Excitation/Emission Max	553/568 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2535844

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	1-10 μg/mL	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	2 μg/mL	0 Publication
Flow Cytometry (Flow)	1-10 μg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

#### **Product Specific Information**

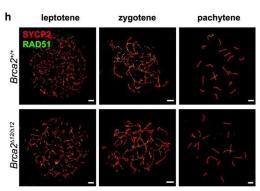
To minimize cross-reactivity, these goat anti-mouse IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG and human serum prior to conjugation. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 555 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

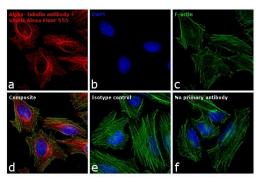
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10  $\mu$ g/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

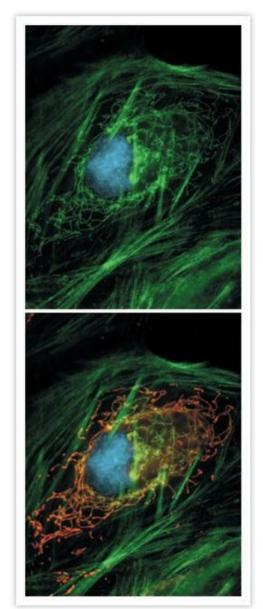
## Product Images For Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in ICC/IF Meiotic phenotype of Brca2 exon 12 deletion mouse model.a Schematic depiction of the domain composition of the BRCA2 protein and the exons 11-15 encoding HSF2BP-binding and DMC1-binding domains. Introns are not drawn to scale; different exon phases are indicated by the shape of the boundary. Location of Cas9 cut sites for exon 12 and exons 12-14 excision is shown. b RT-PCR on cDNA from mouse testis with indicated genotypes confirming the loss of exon 12; primer locations are shown on the exon scheme in a. The PCR was performed twice with the same results. c Immunoblot analysis of proteins precipitated from Brca2+/+ and Brca212/12 mouse testes using anti-HSF2BP antibody and, as control, with anti-RAD51 antibodies; and the input samples; performed as described in "Methods" section. The experiment was performed four times with similar results. d testis weight, e bodyweight, f sperm count, and g epididymis weight in Brca212/12 and control mice. n = 5 animals for Brca2+/+, n = 6 for Brca212/12, n = 3 (testis weight and bodyweight), and n = 2 (epididymis weight and sperm count) for Brca212/+. Mean, s.e.m, and p values from one-way ANOVA with Tukey test are indicated. h-n Immunofluorescent analysis of meiotic protein localization on spermatocyte spreads from Brca212/12 and control mice. Representative images (h, l; scale bars = 5 µm) and quantificatio... Image collected and cropped by CiteAb from the following publication (https://pubmed. ncbi.nlm.nih.gov/34326328), licensed under a CC BY license.



Mouse IqG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in ICC/IF Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal Antibody (Product # A11126). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 μg/mL primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 (Product # A-21422) was used at a concentration of 2 μg/mL in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in ICC/IF HeLa cell transfected with pShooter pCMV/myc/mito/GFP, then fixed and permeabilized. Green-fluorescent protein (GFP) localized in the mitochondria was labeled with anti-GFP mouse IgG<br/>sub>2a</sub> (Product # A-11120) and detected with orange-fluorescent Alexa Fluor® 555 goat anti-mouse IgG (Product # A-21422), which colocalized with the dim GFP fluorescence. F-actin was labeled with green-fluorescent Alexa Fluor® 488 phalloidin (Product # A12379), and the nucleus was stained with blue-fluorescent DAPI (Product # D1306, D3571, D21490). The sample was mounted using ProLong® Gold antifade reagent (Product # P36930). Some GFP fluorescence is retained in the mitochondria after fixation (top), but immunolabeling and detection greatly improve visualization (bottom).

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#### **□ 1074 References**

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