# abcam

### Product datasheet

### Anti-Granzyme B antibody ab4059

pH: 7.60

Polyclonal

lgG

Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Immunogen affinity purified

### \*\*\*\*\* 21 Abreviews 116 References 2 Images

Ove	view
Over	VIEW

Product name	Anti-Granzyme B antibody	
Description	Rabbit polyclonal to Granzyme B	
Host species	Rabbit	
Specificity	The immunogen used for this product shares 94% homology with Granzyme H. Cross-reactivity with this protein has not been confirmed experimentally.	
Tested applications	Suitable for: IHC-P	
Species reactivity	Reacts with: Human	
	Predicted to work with: Mouse, Rat, Pig, Common marmoset	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
General notes	This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.	
	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.	
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As	
Properties		
Form	Liquid	
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.	

Purity

Storage buffer

Clonality Isotype

1

### Applications

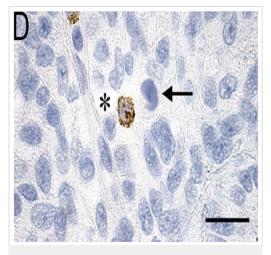
**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab4059 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★ (20)	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target	
Function	This enzyme is necessary for target cell lysis in cell-mediated immune responses. It cleaves after Asp. Seems to be linked to an activation cascade of caspases (aspartate-specific cysteine proteases) responsible for apoptosis execution. Cleaves caspase-3, -7, -9 and 10 to give rise to active enzymes mediating apoptosis.
Sequence similarities	Belongs to the peptidase S1 family. Granzyme subfamily. Contains 1 peptidase S1 domain.
Cellular localization	Cytoplasmic granule. Cytoplasmic granules of cytolytic T-lymphocytes and natural killer cells.

### Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-

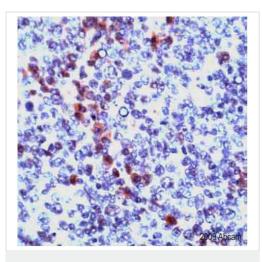
embedded sections) - Anti-Granzyme B antibody

#### (ab4059)

Huszthy et al PLoS One. 2015 Aug 20;10(8):e0136089. doi: 10.1371/journal.pone.0136089. eCollection 2015. Fig 5. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

# Cytotoxic effector cells and regulatory T cells in human glioblastoma xenograft growing in immunocompetent rats.

Granzyme B-positive cytotoxic cells (ab4059 at a 1/75 dilution) in a GBM xenograft with rejection. Asterisks mark tissue lyzed by effector cells.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Granzyme B antibody (ab4059)

Human tonsil tissue stained with anti-Granzyme B antibody (ab4059).

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### Product datasheet

## Anti-PD1 antibody [NAT105] ab52587

### \*\*\*\*\* 9 Abreviews 192 References 14 Images

### Overview

Product name	Anti-PD1 antibody [NAT105]		
Description	Mouse monoclonal [NAT105] to PD1		
Host species	Mouse		
Tested applications	Suitable for: WB, Flow Cyt, IHC-Fr, ICC/IF, IHC-P		
Species reactivity	Reacts with: Human		
Immunogen	Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers.		
Positive control	IHC-P: Human tonsil tissue. ICC/IF: MOLT-4 cells. Flow Cyt: Human tonsil extract. MOLT-4 cells. IHC-Fr: Human tonsil tissue.		
General notes	Please note that PD-1 is expressed variably in different tissues and that optimisation may be required depending on the tissue used for the experiment.		
	Western blot protocol advice:		
	Due to low expression of PD-1, we recommend loading a high amount of sample (100 $\mu$ g) to detect the band for PD-1. Human tonsil and YT cell line lysates are suitable positive controls.		
	New alternative versions of the NAT105 clone available: Recombinant version (ab234444) PBS-only recombinant version (ab201811) Chimeric recombinant rabbit version (ab216352)		
	To test multiple PD-1 monoclonal antibodies please see our PD-1 trial-size panel – ab252192		
	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.		
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As		

### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or - 80°C. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	NAT105
lsotype	lgG1
Light chain type	kappa

### Applications

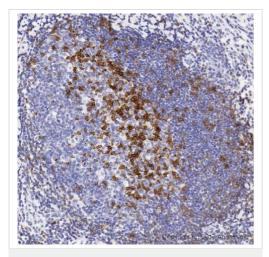
The Abpromise guarantee Our Abpromise guarantee covers the use of ab52587 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/50. Predicted molecular weight: 32 kDa.
Flow Cyt		1/100. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
IHC-Fr	★★★★☆ (1)	Use at an assay dependent concentration.
ICC/IF		Use a concentration of 5 - 10 µg/ml. We recommend Goat Anti-Mouse IgG H&L (Alexa Fluor <sup>®</sup> 488) (ab150117) secondary antibody.
IHC-P	****(7)	1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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Function	Possible cell death inducer, in association with other factors.
Involvement in disease	Genetic variation in PDCD1 is associated with susceptibility to systemic lupus erythematosus type 2 (SLEB2) [MIM:605218]. Systemic lupus erythematosus is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. It is thought to represent a failure of the regulatory mechanisms of the autoimmune system.
Sequence similarities	Contains 1 lg-like V-type (immunoglobulin-like) domain.
Developmental stage	Induced at programmed cell death.
Cellular localization	Membrane.

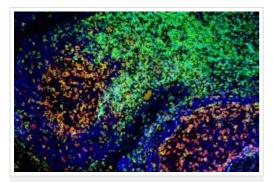


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

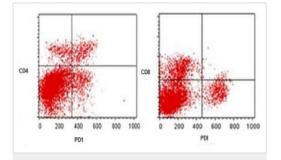
IHC image of PD1 staining in normal human tonsil formalin fixed paraffin embedded tissue section\*, performed on a Leica Bond<sup>TM</sup> system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab52587 at 5 µg/ml for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with hematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

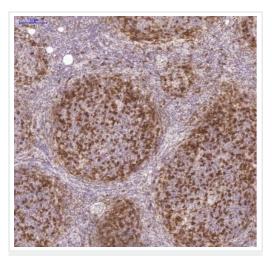


Flow Cytometry - Anti-PD1 antibody [NAT105] (ab52587)

Double immunofluorescence staining of CD3 (green) and PD1 (red) on paraffin embedded tonsil.

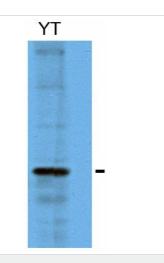
Sample: Human tonsil cell extract.

Dilution: ab52587 antibody was used as 1/200 in 1x10<sup>6</sup> cells/tube. Anti-CD4 antibody was used as 1/200 dilution. Anti-CD8 antibody was used as 1/200 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

Image courtesy of an AbReview submitted by Dr Hajnalka Rajnai



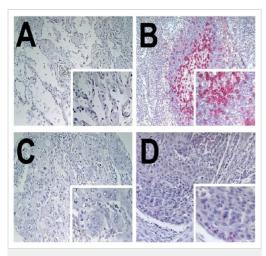
Western blot - Anti-PD1 antibody [NAT105] (ab52587)

This image is courtesy of an anonymous collaborator.

Formaldehyde-fixed, paraffin-embedded human follicular lymphoma tissue stained for PD1 with ab52587 at 1/100 dilution in immunohistochemical analysis.

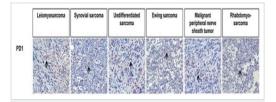
Anti-PD1 antibody [NAT105] (ab52587) at 1/50 dilution + YT cell line extracts

Predicted band size: 32 kDa Observed band size: 47 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

Schmidt et al PLoS One. 2015 Aug 27;10(8):e0136023. doi: 10.1371/journal.pone.0136023. eCollection 2015. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Immunohistochemistry (Formalin/PFA-fixed paraffin-

embedded sections) - Anti-PD1 antibody [NAT105]

#### (ab52587)

Kim et al PLoS One. 2013 Dec 11;8(12):e82870. doi: 10.1371/journal.pone.0082870. eCollection 2013. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/ Representative immunohistochemical staining results for PD1 using ab52587 at a 1/50 dilution in human formalin-fixed, paraffin-embedded tissue specimens.

Panel A: Normal lung tissue, negative control;

Panel B: Tonsillar tissue, positive control;

Panel C: PD1-negative tumor infiltrating lymphocytes;

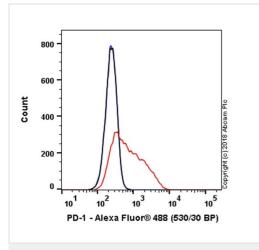
Panel D: PD1-positive tumor infiltrating lymphocytes in squamous cell carcinomas).

Image from PMID: 26313362

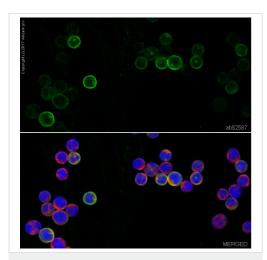
Immunohistochemical analysis of various soft tissue sarcomas staining PD1 using ab52587 at a 1/50 dilution.

Arrows indicate PD1 positive lymphocytes.

Image from PMID: 24349382



Flow Cytometry - Anti-PD1 antibody [NAT105] (ab52587)



Immunocytochemistry/ Immunofluorescence - Anti-PD1 antibody [NAT105] (ab52587)

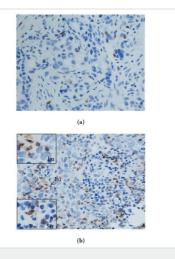
Overlay histogram showing MOLT-4 (Human lymphoblastic leukemia cell line) cells stained with ab52587 (red line). Live cells were incubated in 1x PBS / 10% normal goat serum to block nonspecific protein-protein interactions followed by the antibody (ab52587, 1/100 dilution) for 30 min at 4°C. The secondary antibody used was Alexa Fluor<sup>®</sup> 488 goat anti-mouse lgG (H&L) (ab150117) at 1/2000 dilution for 30 min at 4°C.

A mouse IgG1 isotype control antibody (ab170190) was used at the same concentration and conditions as the primary antibody (black line). Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

MOLT-4 (Human lymphoblastic leukemia cell line) cells stained for PD1 (colored green) using ab52587 in ICC/IF.

Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% Tween-20 for 1 hour at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with ab52587 at 10  $\mu$ g/ml and ab6046 (Rabbit polyclonal to beta Tubulin - Loading Control) at 1  $\mu$ g/ml overnight at +4°C. The secondary antibodies used were Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150117) secondary antibody used at 1  $\mu$ g/ml (colored green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 594) (ab150080) secondary antibody (pseudo-colored red) used at 2  $\mu$ g/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43  $\mu$ M for 1 hour at room temperature.

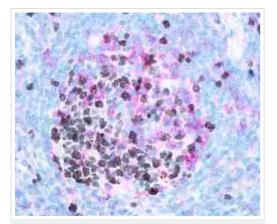


Immunohistochemistry (Formalin/PFA-fixed paraffin-

embedded sections) - Anti-PD1 antibody [NAT105]

#### (ab52587)

This image is from PubMedId: 27777963. Kaewkangsadan V et al. (2016) Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/



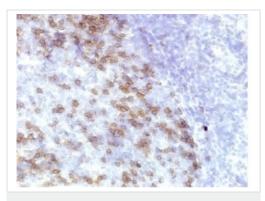
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587) Immunohistochemical analysis of human large and locally advanced breast cancers staining PD1 using ab52587.

(Panel a) Low level of PD-1<sup>+</sup> T cell infiltration.

(Panel b) high level of PD-1<sup>+</sup> T cell infiltration. (Itu: intratumoral Str: stromal).

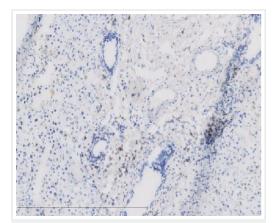
Image data from PMID: 27777963

Double immunoenzymatic staining of Ki67 (brown) and PD1 (red) on paraffin embedded tonsil.

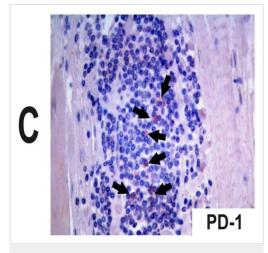


Immunohistochemistry (Frozen sections) - Anti-PD1 antibody [NAT105] (ab52587)

Human tonsil tissue stained for PD1 with ab52587 incubated for 30 mins at a 1/100 dilution in immunohistochemical analysis.



Immunohistochemistry (Frozen sections) - Anti-PD1 antibody [NAT105] (ab52587) This image is courtesy of an anonymous Abreview Immunohistochemical analysis of frozen human liver tissue labeling PD1 with ab52587 at 1/50 dilution.



Expression of markers of T cell differentiation and degree of inflammation in the heart of chronically *T. cruzi*-infected subjects with severe cardiomyopathy.

A–D, left panel: representative photos of CD45RO, CD27, PD1 and CD57 expression, respectively.

Panel C shown.

Image data from PMID: 25144227

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

Argüello et al PLoS Negl Trop Dis. 2014 Aug 21;8(8):e2989. doi: 10.1371/journal.pntd.0002989. eCollection 2014 Aug. Fig 2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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# abcam

### Product datasheet

## Anti-CD4 antibody [EPR6855] ab133616

Recombinant RabMAb

\*\*\*\*\* 17 Abreviews 154 References 19 Images

### Overview

Product name	Anti-CD4 antibody [EPR6855]		
Description	Rabbit monoclonal [EPR6855] to CD4		
Host species	Rabbit		
Tested applications	Suitable for: Flow Cyt (Intra), ICC, WB, IHC-P		
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat		
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.		
Positive control	WB: THP-1 and HuT-78 cell lysates, human fetal thymus, tonsil and lymph node tissue lysates. IHC-P: Human tonsil, liver, spleen, thymoma and colon tissues. ICC: Human peripheral blood lymphocytes and THP-1 cells. Flow Cyt (intra): Human peripheral blood lymphocytes.		
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information see here.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb<sup>®</sup> patents.</li> </ul>		
	We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.		

### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6855
Isotype	lgG

### Applications

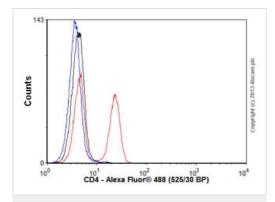
The Abpromise guarantee Our Abpromise guarantee covers the use of ab133616 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

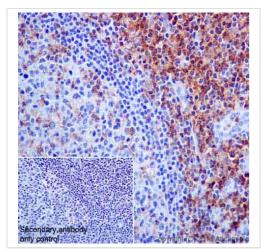
Application	Abreviews	Notes
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC		1/100 - 1/250.
WB		1/5000. Detects a band of approximately 51 kDa (predicted molecular weight: 51 kDa). For unpurified use at 1/1000 - 1/10000.
IHC-P	★★★★ (13)	1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
		For unpurified use at 1/100 - 1/250.

Target	
Function	Accessory protein for MHC class-II antigen/T-cell receptor interaction. May regulate T-cell activation. Induces the aggregation of lipid rafts.
Sequence similarities	Contains 3 lg-like C2-type (immunoglobulin-like) domains. Contains 1 lg-like V-type (immunoglobulin-like) domain.
Post-translational modifications	Palmitoylation and association with LCK contribute to the enrichment of CD4 in lipid rafts.
Cellular localization	Cell membrane. Localizes to lipid rafts. Removed from plasma membrane by HIV-1 Nef protein that increases clathrin-dependent endocytosis of this antigen to target it to lysosomal degradation. Cell surface expression is also down-modulated by HIV-1 Envelope polyprotein gp160 that interacts with, and sequesters CD4 in the endoplasmic reticulum.

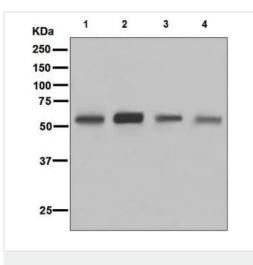
### Images



Flow Cytometry (Intracellular) - Anti-CD4 antibody [EPR6855] (ab133616) Human peripheral blood lymphocytes stained with unpurifiedab133616 (red line). Human whole blood was processed using a modified protocol based on Chow et al, 2005 (PMID: 16080188). In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 min at 22°C. Red blood cells were then lyzed by the addition of Triton X-100 (final concentration -0.1%) for 15 min at 37°C. For experimentation, cells were treated with 50% methanol (-20°C) for 15 min at 4°C. Cells were then incubated with the antibody (unpurified ab133616, 1/100 dilution) for 30 min at 4°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was rabbit IgG (monoclonal)  $(0.1\mu g/1x10^6$  cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >30,000 total events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. Gating strategy - peripheral blood lymphocytes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with purified ab133616 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-CD4 antibody [EPR6855] (ab133616)

All lanes : Anti-CD4 antibody [EPR6855] (ab133616) at 1/1000 dilution (unpurified)

Lane 1 : THP-1 cell lysate Lane 2 : Human fetal thymus lysate Lane 3 : Human tonsil lysate Lane 4 : Human lymph node lysate

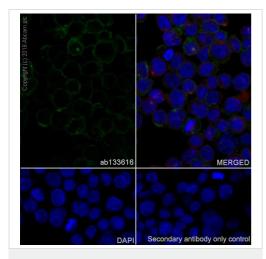
Lysates/proteins at 10 µg per lane.

### Secondary

Lane 1 : HRP labelled goat anti-rabbit at 1/2000 dilution Lanes 2-4 : HRP labelled goat anti-rabbit at 1/2000 dilution

Immunocytochemistry analysis of THP-1 (Human monocytic

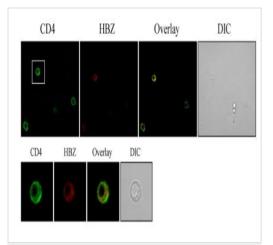
Predicted band size: 51 kDa Observed band size: 51 kDa



dilution. Cells were fixed with 100% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.30 µg/ml) was used as counterstain. Nuclei were stained blue with DAPI. Negative control: PBS instead of the primary antibody.

leukemia monocyte) labeling CD4 with purified ab133616 at 1/100

Immunocytochemistry - Anti-CD4 antibody [EPR6855] (ab133616)



#### Immunocytochemistry - Anti-CD4 antibody

#### [EPR6855] (ab133616)

Baratella et al PLoS Negl Trop Dis. 2017 Jan; 11(1): e0005285. Published online 2017 Jan 17. doi: 10.1371/journal.pntd.0005285

## HBZ is preferentially expressed in CD4+ T cells of HAM/TSP patient PH1624

Confocal microscopy analysis of PBMC from HAM/TSP patient PH1624. (A) co-staining with the 4D4-F3 anti-HBZ mAb followed by Alexa Fluor 546-conjugated goat anti-mouse IgG1 antibody (red) and with the anti-CD4 mAb followed by Alexa Fluor 488-conjugated goat-anti-rabbit IgG antibody (green); upper panels, extended field; lower panels, enlarged field focused on the single cell depicted in the square of the left upper panel and positive for both CD4 and HBZ.

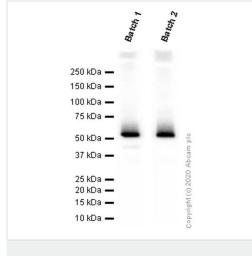
CD4 was detected using ab133616 at 1/100 dilution.

From Figure 6A of Baratella et al.

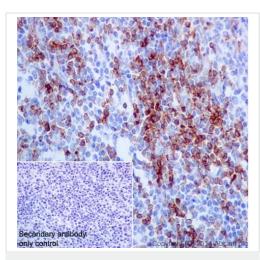
Baratelle et al PLoS Negl Trop Dis. 2017 Jan; 11(1): e0005285. Published online 2017 Jan 17. doi: 10.1371/journal.pntd.0005285

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Different batches of ab133616 were tested on THP-1 (Human monocytic leukemia monocyte) lysate at 1.0  $\mu$ g/ml. 15  $\mu$ g of lysate was loaded in each lane. Bands observed at 51 kDa.

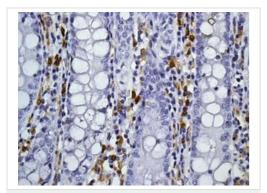


Western blot - Anti-CD4 antibody [EPR6855] (ab133616)



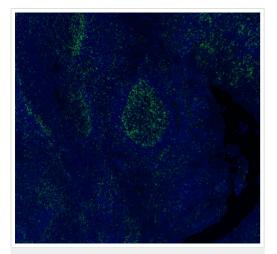
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thymoma tissue labelling CD4 with purified ab133616 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling CD4 with ab133616 at a dilution of 1/100.

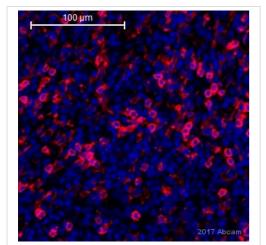
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

#### Anti-CD4 antibody [EPR6855] (ab133616)

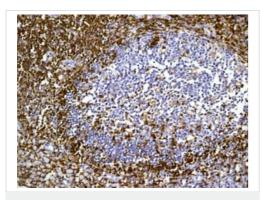
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with ab133616 at a dilution of 1:500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via Phenochart software. This image was courteously provided by Dr. Houssein Abdul Sater, Georgia Cancer Center.



Paraffin-embedded human spleen tissue stained for CD4 using ab133616 at 1/500 dilution in immunohistochemical analysis.

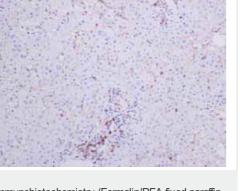
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

This image is courtesy of an anonymous Abreview.



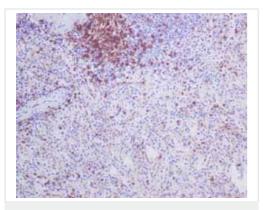
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD4 with unpurified ab133616 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling CD4 with unpurified ab133616.

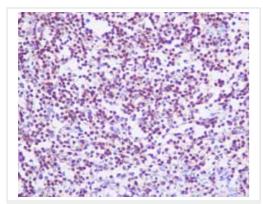
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

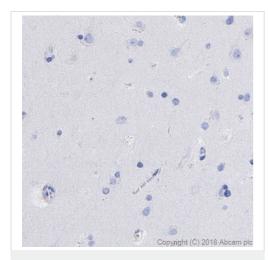
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling CD4 with unpurified ab133616.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thymoma tissue labelling CD4 with unpurified ab133616.

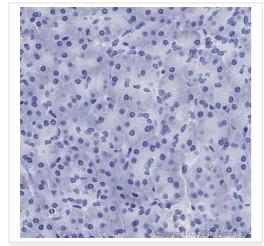
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616)

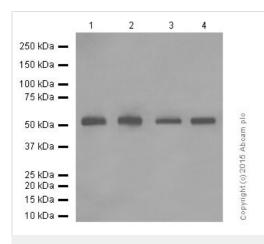
Negative control: no staining on human cerebrum.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum showing no staining CD4 with purified ab133616 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (ab93684). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Negative control: no staining on human pancreas.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreas showing no staining CD4 with purified ab133616 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 (ab93684). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Counterstained with hematoxylin.



Western blot - Anti-CD4 antibody [EPR6855]

(ab133616)

**All lanes :** Anti-CD4 antibody [EPR6855] (ab133616) at 1/5000 dilution (purified)

Lane 1 : Human fetal thymus tissue lysate
Lane 2 : Human tonsil tissue lysate
Lane 3 : THP-1 cell lysate
Lane 4 : HuT-78 cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

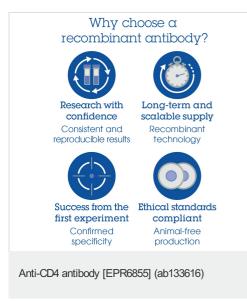
**All lanes :** HRP-conjugated anti-rabbit IgG, specific to the nonreduced form of IgG at 1/1000 dilution

Predicted band size: 51 kDa Observed band size: 51 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

		T	issue Microarray (IN	(A) data for ab1336	16		
	Normal fiss	ue samples			Malignant ti	ssue samples	
Human cardiac muscle	x	Human placenta	×	Clear cell carcinoma of human kidney	×	Human liver carcinoma	1
luman cerebrum	x	Human skeletal muscle	x	Human bladder cancer	¥ [immune cels ✔]	Human lung carcinoma	x
Human colon	* (mmune cells 🗸 )	Human skin	×	Human breast carcinoma	¥ [mmune cels √]	Human melanoma	*
Human endometrium	× (mmune cels ✓)	Human spleen	1	Human cervical carcinoma	¥ [mmune cels ✔]	Human non- Hodgkin's lymphoma	✓
Human kidney	x	Human stomach	× (immune cells 🗸 )	Human colon carcinoma	× [immune cells √]	Human ovarian carcinoma	x
Human liver	× (mmune cels ✓)	Human testis	×	Human endometrial carcinoma	¥ [mmune cels ✔]	Human prostatic hyperplasia	×
Human lung	x	Human thyroid	×	Human gastric adenocarcinoma	¥ [immune cels ✔]	Human thymoma	1
luman mammary gland	× (immune cells ✔)	Human tonsil	1	Human glioma	×	Human thymus hyperplasia	1
iuman pancreas	×			Human hepatocellular carcinoma	×	Human thyroid carcinoma	× (immune cells v
				Human Hodgkin's lymphoma	1		

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD4 antibody [EPR6855] (ab133616) Tissue Microarrays stained for "Anti-CD4 antibody [EPR6855]" using "ab133616" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond<sup>™</sup> Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab133616 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument.



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### Product datasheet

## Anti-CD1c antibody [OTI2F4] ab156708

### \*\*\*\* 4 Abreviews 7 References 4 Images

### Overview

Product name	Anti-CD1c antibody [OTI2F4]
Description	Mouse monoclonal [OTI2F4] to CD1c
Host species	Mouse
Tested applications	Suitable for: WB, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Recombinant full length protein corresponding to CD1c. produced in HEK293T cell
General notes	The clone number has been updated from 2F4 to OTI2F4, both clone numbers name the same clone.
	This product was changed from ascites to tissue culture supernatant on 29 <sup>th</sup> May 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.
	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.30 Preservative: 0.02% Sodium azide Constituents: PBS, 1% BSA, 50% Glycerol
Purity	Protein G purified
Purification notes	Purified from TCS
Clonality	Monoclonal
Clone number	OTI2F4

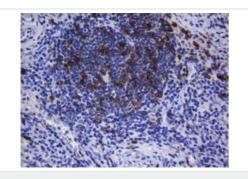
### Applications

The Abpromise guaranteeOur Abpromise guarantee covers the use of ab156708 in the following tested applications.The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

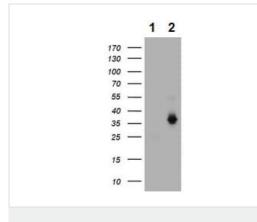
Application	Abreviews	Notes
WB		1/2000. Predicted molecular weight: 38 kDa.
IHC-P	$\star$ $\star$ $\star$ $\star$ $\star$ (4)	1/150.

Target	
Function	Antigen-presenting protein that binds self and non-self lipid and glycolipid antigens and presents them to T-cell receptors on natural killer T-cells.
Tissue specificity	Expressed on cortical thymocytes, on certain T-cell leukemias, and in various other tissues.
Sequence similarities	Contains 1 lg-like (immunoglobulin-like) domain.
Cellular localization	Cell membrane. Endosome membrane. Subject to intracellular trafficking between the cell membrane and endosomes.

### Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD1c antibody [OTI2F4] (ab156708) Immunohistochemical staining of paraffin-embedded Human lymph node tissue using anti-CD1C mouse monoclonal antibody(ab156708)



Western blot - Anti-CD1c antibody [OTI2F4] (ab156708)

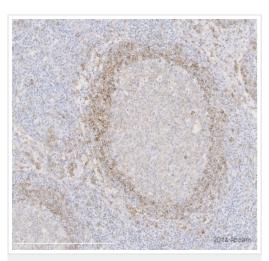
All lanes : Anti-CD1c antibody [OTI2F4] (ab156708)

Lane 1 : HEK293T cells were transfected with the pCMV6-ENTRY Lane 2 : HEK293T cells were transfected with pCMV6-ENTRY CD1C

Lysates/proteins at 5 µg per lane.

Predicted band size: 38 kDa

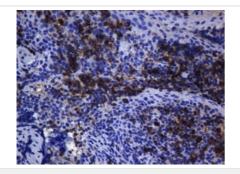
HEK293T cell lysates were generated from transient transfection of the cDNA clone (RC211378)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD1c antibody [OTI2F4] (ab156708)

This image is courtesy of an Abreview submitted by Jing Ma

ab156708 staining MAFA in Cynomolgus monkey lymph node sections by Immunohistochemistry (IHC-P paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% donkey serum for 20 minutes at romm temperature; antigen retrieval was by heat mediation. Samples were incubated with primary antibody (1/50) for 30 minutes at room temperature. A Biotin-conjugated Donkey anti-mouse IgG polyclonal (1/2000) was used as the secondary antibody.



Immunohistochemical staining of paraffin-embedded Human tonsil using anti-CD1C mouse monoclonal antibody. (ab156708)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD1c antibody [OTI2F4] (ab156708)

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### Product datasheet

## Anti-CD163 antibody [EPR19518] ab182422

Recombinant RabMAb

### \*\*\*\*\* 15 Abreviews 171 References 24 Images

### Overview

Product name	Anti-CD163 antibody [EPR19518]
Description	Rabbit monoclonal [EPR19518] to CD163
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt, IHC-P, WB, IHC-Fr
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human fetal liver, spleen and tonsil lysates; Mouse and rat liver, heart, spleen and thymus lysates; IHC-P: Human liver, tonsil and placenta tissue.; human breast carcinoma tissue; Mouse liver and spleen tissue. Rat liver, achilles and muscle tissues; IHC-Fr: Mouse spleen and liver tissues. Flow Cyt: Human PBMC cells. ICC: SU-DHL-1 cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information see here.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb<sup>®</sup> patents.</li> </ul>

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19518

### Applications

**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab182422 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/60.
IHC-P	<b>★ ★ ★ ★ ★ (13)</b>	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	<b>★★★★</b> (1)	1/1000. Detects a band of approximately 150 kDa (predicted molecular weight: 121 kDa).
IHC-Fr		1/200. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).

### Target

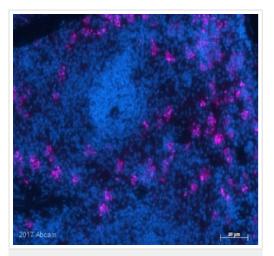
Function	Acute phase-regulated receptor involved in clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages and may thereby protect tissues from free hemoglobin-mediated oxidative damage. May play a role in the uptake and recycling of iron, via endocytosis of hemoglobin/haptoglobin and subsequent breakdown of heme. Binds hemoglobin/haptoglobin complexes in a calcium-dependent and pH-dependent manner. Exhibits a higher affinity for complexes of hemoglobin and multimeric haptoglobin of HP*1F phenotype than for complexes of hemoglobin and dimeric haptoglobin of HP*1S phenotype. Induces a cascade of intracellular signals that involves tyrosine kinase-dependent calcium mobilization, inositol triphosphate production and secretion of IL6 and CSF1. Isoform 3 exhibits the higher capacity for ligand endocytosis and the more pronounced surface expression when expressed in cells. After shedding, the soluble form (sCD163) may play an anti-inflammatory role, and may be a valuable diagnostic parameter for monitoring macrophage activation in inflammatory conditions.
Tissue specificity	Expressed in monocytes and mature macrophages such as Kupffer cells in the liver, red pulp macrophages in the spleen, cortical macrophages in the thymus, resident bone marrow macrophages and meningeal macrophages of the central nervous system. Expressed also in blood. Isoform 1 is the lowest abundant in the blood. Isoform 2 is the lowest abundant in the liver and the spleen. Isoform 3 is the predominant isoform detected in the blood.
Sequence similarities	Contains 9 SRCR domains.
Domain	The SRCR domain 3 mediates calcium-sensitive interaction with hemoglobin/haptoglobin complexes.
Post-translational modifications	A soluble form (sCD163) is produced by proteolytic shedding which can be induced by lipopolysaccharide, phorbol ester and Fc region of immunoglobulin gamma. This cleavage is dependent on protein kinase C and tyrosine kinases and can be blocked by protease inhibitors. The shedding is inhibited by the tissue inhibitor of metalloproteinase TIMP3, and thus probably induced by membrane-bound metalloproteinases ADAMs.

### Phosphorylated.

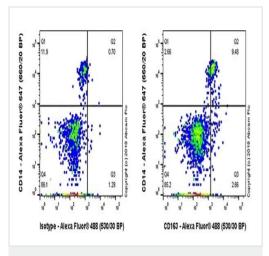
### **Cellular localization**

Secreted and Cell membrane. Isoform 1 and isoform 2 show a lower surface expression when expressed in cells.

### Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) Image courtesy of an anonymous Abreview.

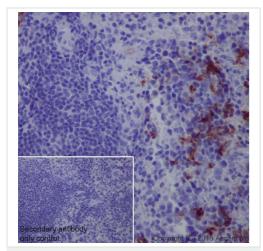


Flow Cytometry - Anti-CD163 antibody [EPR19518] (ab182422) 10% NBF, non-permeabilized mouse spleen tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor<sup>®</sup>647 conjugate was used as the secondary at a 1/200 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Sodium citrate pH 6.0.

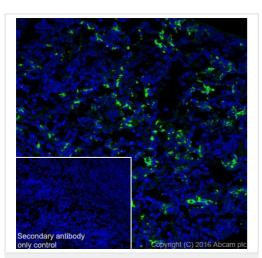
Blocking step: 5% serum for 1 hour at 21°C.

Flow cytometry analysis of human PBMC cells (Human peripheral blood mononuclear cell) labeling with ab182422 at 1/60 dilution, 11.23 µg/ml (red). Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, ab150081) was used as the secondary antibody at 1/2000.



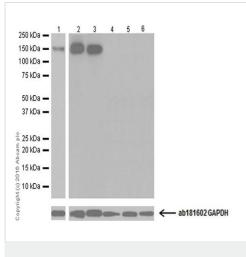
Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on mouse spleen is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)



Immunohistochemistry (Frozen sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse spleen tissue labeling CD163 with ab182422 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result showed some cytoplasmic staining on mouse spleen. The nuclear counterstain is DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 at 1/1000 dilution. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).



Western blot - Anti-CD163 antibody [EPR19518] (ab182422)

All lanes : Anti-CD163 antibody [EPR19518] (ab182422) at 1/1000 dilution

- Lane 1 : Human fetal liver lysate
- Lane 2 : Human tonsil lysate
- Lane 3 : Human fetal spleen lysate

Lane 4 : U937 (Human histiocytic lymphoma cell line) whole cell lysate

Lane 5 : THP-1 (Human monocytic leukemia cell line) whole cell lysate

Lane 6 : J774A.1 (Mouse macrophage reticulum cell sarcoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 121 kDa Observed band size: 150 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 1 minute; Lane 2-5: 3 minutes.

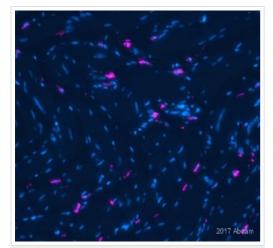
U937, THP-1 and J774A.1 cell lines were reported to be negative for CD163 expression.(PMID:16368951, 10648003 & 10577520).

The molecular weight observed is consistent with what has been described in the literature (PMID:9712057 & 16517975).



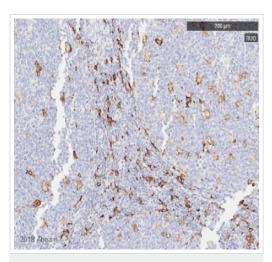
Western blot - Anti-CD163 antibody [EPR19518] (ab182422)

Different batches of ab182422 were tested on Rat liver lysate at 2.0  $\mu$ g/ml. 15  $\mu$ g of lysate was loaded in each lane. Bands observed at 150 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) This image is courtesy of an anonymous Abreview. 10% NBF, non-permeabilized rat muscle tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor<sup>®</sup>647 conjugate was used as the secondary (red). Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

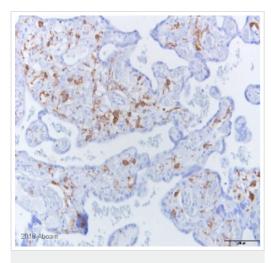
Blocking step: 5% serum for 1 hour at 22°C.



Formaldehyde-fixed, non-permeabilized human tonsil tissue stained for CD163 with ab182422 (30 mins at a 1/400 dilution) in immunohistochemical analysis. A Goat polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 9.0 EDTA. Blocking step: 1% ab64226 for 10 mins at RT.

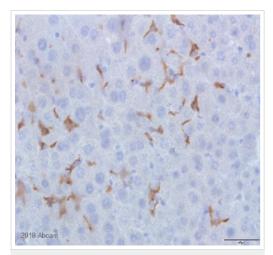
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) Image courtesy of an anonymous Abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) This image is courtesy of an anonymous Abreview. Formaldehyde-fixed, non-permeabilized human placenta tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Goat anti Rabbit polyclonal HRP conjugate was used as the secondary at a 1/200 dilution.

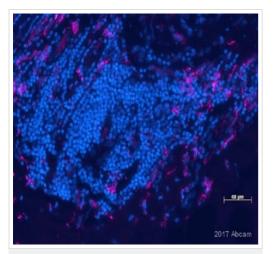
Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate buffer.

Blocking step: 3% serum for 30 mins at 20°C.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

This image is courtesy of an anonymous Abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) This image is courtesy of an anonymous Abreview. Formaldehyde-fixed, non-permeabilized mouse liver tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Goat anti Rabbit HRP conjugate was used as the secondary at a 1/200 dilution.

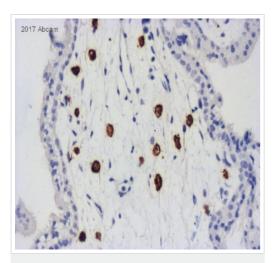
Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate buffer.

Blocking step: 3% serum for 30 mins at 20°C.

10% Formalin-fixed, non-permeabilized human breast carcinoma tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor<sup>®</sup>647 conjugate was used as the secondary at a 1/200 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 5% serum for 1 hour at 22°C.



Immunohistochemistry (Formalin/PFA-fixed paraffin-

embedded sections) - Anti-CD163 antibody

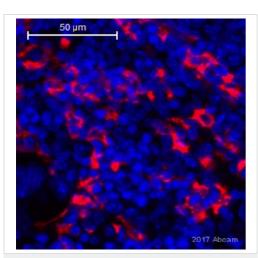
This image is courtesy of an anonymous Abreview.

[EPR19518] (ab182422)

Formaldehyde-fixed, non-permeabilized human first trimester placenta tissue stained for CD163 with ab182422 (16 hours, 4°C at a 1/250 dilution) in immunohistochemical analysis. A Pig anti Rabbit polyclonal biotin conjugate was used as the secondary at a 1/250 dilution.

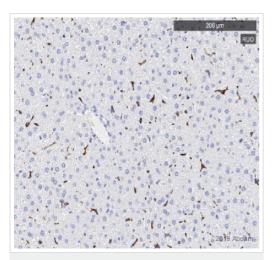
Heat mediated antigen retrieval buffer/enzyme used: Sodium citrate.

Blocking step: 5% BSA for 30 mins at 22°C.



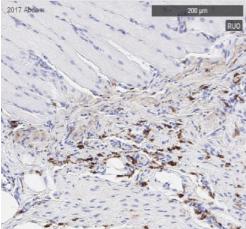
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) This image is courtesy of an anonymous Abreview. 10% NBF-fixed mouse spleen tissue stained for CD163 with ab182422 (18 hours at a 1/250 dilution) in immunohistochemical analysis. A Goat anti Rabbit IgG polyclonal AlexaFluor<sup>®</sup>647 conjugate was used as the secondary at a 1/600 dilution (red). Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 20% serum for 1 hour at RT.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

This image is courtesy of an anonymous Abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) This image is courtesy of an anonymous Abreview.

200 µm Formaldehyde-fix

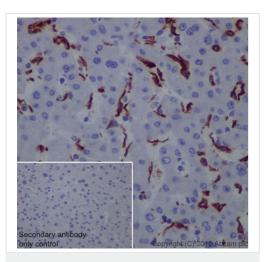
Formaldehyde-fixed, non-permeabilized mouse liver tissue stained for CD163 with ab182422 (45 mins at a 1/400 dilution) in immunohistochemical analysis. A Rabbit polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate. Blocking step: 1% ab64226 for 10 mins at RT.

Formaldehyde-fixed, non-permeabilized rat achilles tissue stained for CD163 with ab182422 (30 mins at a 1/200 dilution) in immunohistochemical analysis. A Rabbit polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate 70°C for 2hrs.

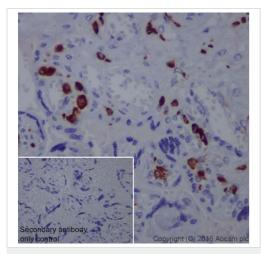
Blocking step: 1% ab64226 for 10 mins at RT.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of human liver is observed. Counter stained with Hematoxylin.

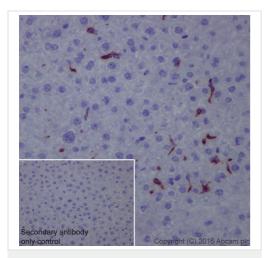
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Hofbauer cells in human placenta is observed. Counter stained with Hematoxylin.

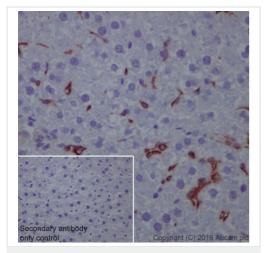
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of mouse liver is observed. Counter stained with Hematoxylin.

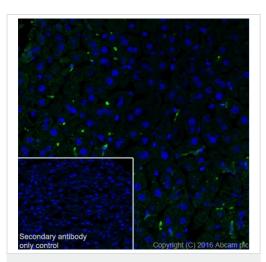
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of rat liver is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Frozen sections) - Anti-CD163 antibody [EPR19518] (ab182422)

5 6

pyright (c) 2016 Abcam plo

250 kDa -

150 kDa -

100 kDa -

75 kDa -

50 kDa 🗕

37 kDa -

25 kDa -

20 kDa -

15 kDa 🗕

10 kDa 🗕

Western blot - Anti-CD163 antibody [EPR19518]

1 2

250 kDa -

150 kDa 🗕

100 kDa -

75 kDa 🗕

50 kDa 🗕

37 kDa -

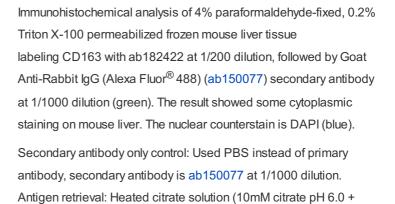
25 kDa -

20 kDa -

15 kDa 🗕

10 kDa 🗕

(ab182422)



All lanes : Anti-CD163 antibody [EPR19518] (ab182422) at 1/1000 dilution

Lane 1 : Mouse liver lysate

0.05% Tween-20).

- Lane 2 : Mouse heart lysate
- Lane 3 : Mouse spleen lysate
- Lane 4 : Mouse thymus lysate
- Lane 5 : Rat liver lysate
- Lane 6 : Rat heart lysate
- Lane 7 : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

# Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 121 kDa Observed band size: 150 kDa

# 13

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID:9712057 & 16517975).

Tissue Microarray (IMA) data for ab182422							
	Mouse normal	fissue samples			Rat normal tis	sue samples	
Viouse cerebrum	I	Mouse pancreas	x	Rat cerebrum	x	Rat pancreas	× (immune cells 🗸
Mouse colon	× (immune cells √)	Mouse skin	× (immune cels √)	Rat colon	¥ [immune cells ✔]	Ratskin	× (immune cells 🗸
Mouse kidney	x	Mouse spleen	1	Ratkidney	¥ [mmune cells ✔]	Rat spleen	1
Mouse liver	× (Kupffer cels √)	Mouse stomach	× (mmune cels 🗸 )	Ratliver	× (Kuptler cells ✔)	Ratistomach	× (immune cells 🗸
Mouselung	× (mmune cells √)	Mouse testis	¥ (immune cels ✔)	Ratilung	≭ (immune cells 🗸 )	Rat testis	× (immune cells 🗸

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) Tissue Microarrays stained for "Anti-CD163 antibody [EPR19518]" using "ab182422" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond<sup>™</sup> Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab182422 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument.

			issue Microarray (TM				
	Human norma	l fissue samples			Human maligna	nt tissue samples	
Human cardiac muscle	¥ (mmune cells ✔)	Human placenta	× (immune cels ✓)	Clear cell carcinoma of human kidney	¥ [mmune cels ✔]	Human glioma	1
luman cerebrum	x	Human skeletal muscle	x	Human bladder cancer	¥ [immune cels √]	Human hepatocellular carcinoma	× (immune cells √)
Human colon	× (mmune cels √)	Human skin	× (immune cels √)	Human breast carcinoma	¥ [immune cels √]	Human lung carcinoma	× (immune cells 🗸 )
Human endometrium	¥ (immune cells ✔)	Human spleen	1	Human cervical carcinoma	¥ [mmune cels ✔]	Human ovarian carcinoma	× (immune cells 🗸 )
Human kidney	* (immune cells 🗸 )	Human stornach	× (immune cels ✓)	Human colon carcinoma	* [mmune cels 🗸 ]	Human pancreatic carcinoma	× (immune cells 🗸
Human liver	× (Kupffer cells ✔)	Human testis	× (immune cells ✓)	Human endometrial carcinoma	¥ [mmune cells ✔]	Human prostatic hyperplasia	× (immune cells √)
Human lung	× (alveolar macrophage√)	Human thyroid	x	Human gastric adenocarcinoma	¥ [immune cels ✔]	Human thyroid carcinoma	× (immune cells 🗸 )
luman mammary gland	× (mmune cels ✓)	Human tonsi	1				
łuman pancreas	× (immune cells √)						

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD163 antibody [EPR19518] (ab182422) Tissue Microarrays stained for "Anti-CD163 antibody [EPR19518]" using "ab182422" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond<sup>™</sup> Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab182422 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument.



Anti-CD163 antibody [EPR19518] (ab182422)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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# Product datasheet

# Anti-CD68 antibody [EPR20545] ab213363

Recombinant RabMAb

# \*\*\*\*\* 3 Abreviews 29 References 13 Images

# Overview

Product name	Anti-CD68 antibody [EPR20545]
Description	Rabbit monoclonal [EPR20545] to CD68
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, ICC/IF, mIHC
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human tonsil, fetal liver and fetal spleen lysates; THP-1 and U937 whole cell lysates. IHC-P: Human tonsil and cervix carcinoma. mIHC: Human liver tissue, human duodenum tissue, human colon tissue. ICC/IF: THP-1 and U937 cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information see here.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb<sup>®</sup> patents.</li> </ul>

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20545

# Applications

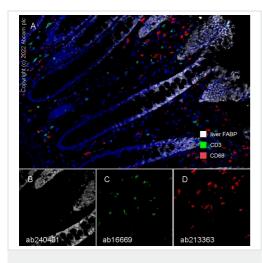
**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab213363 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

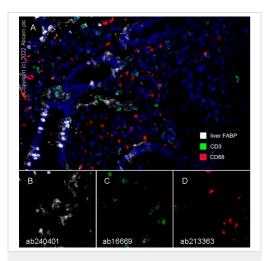
Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 37 kDa).
IHC-P	★★★★ (1)	1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	1/100.
mIHC		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target	
Function	Could play a role in phagocytic activities of tissue macrophages, both in intracellular lysosomal metabolism and extracellular cell-cell and cell-pathogen interactions. Binds to tissue- and organ-specific lectins or selectins, allowing homing of macrophage subsets to particular sites. Rapid recirculation of CD68 from endosomes and lysosomes to the plasma membrane may allow macrophages to crawl over selectin-bearing substrates or other cells.
Tissue specificity	Highly expressed by blood monocytes and tissue macrophages. Also expressed in lymphocytes, fibroblasts and endothelial cells. Expressed in many tumor cell lines which could allow them to attach to selectins on vascular endothelium, facilitating their dissemination to secondary sites.
Sequence similarities	Belongs to the LAMP family.
Post-translational modifications	N- and O-glycosylated.
Cellular localization	Cell membrane and Endosome membrane. Lysosome membrane.

Images



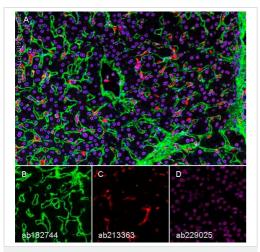
Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] (ab213363)



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] (ab213363)

Fluorescence multiplex immunohistochemical analysis of the human colon (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (ab240401, gray; Opal™690), anti-CD3 (ab16669, green; Opal™520) and anti-CD68 (ab213363, red; Opal<sup>™</sup>570) on human colon. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument with an Opal<sup>™</sup> 4-color kit. The section was incubated in three rounds of staining: in the order of ab240401 (1/8000 dilution), ab16669 (1/150 dilution), and ab213363 (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

Fluorescence multiplex immunohistochemical analysis of the human duodenum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (ab240401, gray; Opal<sup>™</sup>690), anti-CD3 (ab16669, green; Opal<sup>™</sup>520) and anti-CD68 (ab213363, red; Opal<sup>™</sup>570) on human duodenum. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument with an Opal<sup>™</sup> 4-color kit. The section was incubated in three rounds of staining: in the order of ab240401 (1/8000 dilution), ab16669 (1/150 dilution), and ab213363 (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] (ab213363)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363) performed with Leica SP8 confocal microscope.

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) analysis of human liver tissue.

Panel A: Merged staining of Collagen VI (ab182744; green), anti-CD68 (ab213363; red) and anti-Lamin B1 (ab229025; magenta).

Panel B: Anti-Collagen VI (green) stained on extracellular matrix.

Panel C: Anti-CD68 (red) stained on Kupffer cells.

Panel D: Anti-Lamin B1 (magenta) stained on nuclear envelope.

Key protocol steps: The section was incubated in three rounds of staining with ab182744 (1/1000 dilution), ab213363 (1/1000 dilution) and ab229025 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by tyramide signal amplification with the appropriate fluorophore. Heat mediated antigen retrieval was used (Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins after every round of antibody/fluorophore staining.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

DAPI was used as a nuclear counter stain. A ready-to-use anti-Rabbit and Mouse Polymer HRP was used as a secondary. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

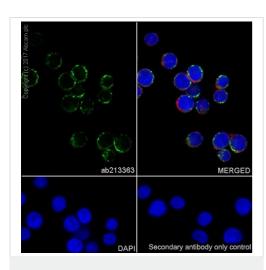
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human spleen tissue labelling PDI with ab243644 at 1.02 µg/mL (B), PD-L 1 with ab213524 at 1/100 dilution (C) and CD68 with ab213363 at 1/300 dilution (D). Anti-Rabbit and Mouse Polymer HRP was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins. Heat mediated antigen retrieval (Leica ER2, PH9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibodies from the previous round, to avoid any cross-reactivity.

Panel A: merged staining of anti- PD1 (green, Opal<sup>™</sup>520), anti-PD-L1 (red, Opal<sup>™</sup>570) and anti- CD68 (yellow, Opal<sup>™</sup>690). Panel B: Anti- PD1 stained on antigen-stimulated T cells. Panel C: anti- PD-L1 stained on cells involved in T cell inhibition Panel D: anti-CD68 stained on macrophages.

The section was incubated in three rounds of staining: in the order

of ab243644, ab213363 and ab213524 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal<sup>™</sup> 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

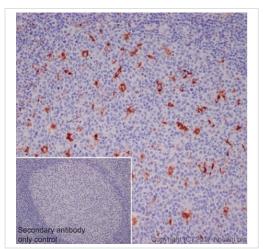


Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] (ab213363)

Immunofluorescent analysis of 100% methanol-fixed THP-1 (human monocytic leukemia cell line) cells labeling CD68 with ab213363 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on THP-1 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (ab195889) (red) at 1/200 dilution.

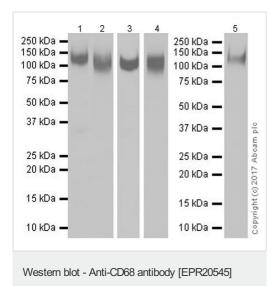
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue, labeling CD68 with ab213363 at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on macrophages of human tonsil is observed (PMID: 19543531). Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.



(ab213363)

Lanes 1-3 & 5 : Anti-CD68 antibody [EPR20545] (ab213363) at 1/1000 dilution

Lane 4 : Anti-CD68 antibody [EPR20545] (ab213363) at 1/5000 dilution

- Lane 1 : Human fetal liver lysate at 20  $\mu$ g
- Lane 2 : Human tonsil lysate at 20  $\mu g$

Lane 3 : Human fetal spleen lysate at 20  $\mu g$ 

Lane 4 : THP-1 (human monocytic leukemia cell line) whole cell lysate at 10  $\mu g$ 

Lane 5 : U937 (human histiocytic lymphoma cell line) whole cell lysate at 10  $\mu g$ 

# Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

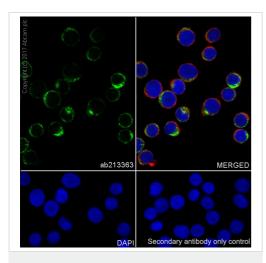
Developed using the ECL technique.

Predicted band size: 37 kDa Observed band size: 110 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2/4/5: 30 seconds; Lane 3: 3 minutes.

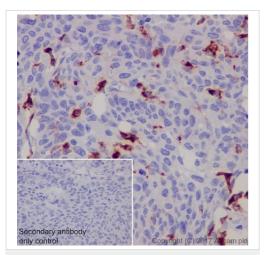
The observed molecular weight is consistent with the literature (PMID:18405323; PMID:11739566; PMID: 16710801).



Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] (ab213363) Immunofluorescent analysis of 100% methanol-fixed U937 (human histiocytic lymphoma cell line) cells labeling CD68 with ab213363 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on U937 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (ab195889) (red) at 1/200 dilution.

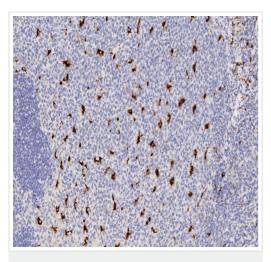
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363)

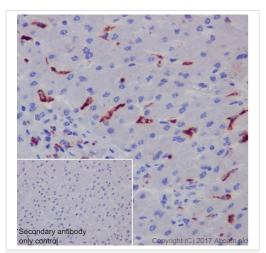
Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue labeling CD68 with ab213363 at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on macrophages of human cervical carcinoma is observed (PMID: 12118106). Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD68 with ab213363 at 1/5000 dilution. No blocking step performed. Anti-Rabbit HRP polymer was used as the secondary detection system. Heat-mediated antigen retrieval was performed using EDTA based pH 9.0 buffer.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CD68 with ab213363 at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on Kupffer cells of human liver is observed (PMID: 12118106). Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.

		T	ssue Microarray (TM	A) data for ab2133	163		
	Normal fiss	ue samples			Malignant fi	sue samples	
Human cardiac muscle	× (mmune cels ✔)	Human placenta	× (immune cels ✔)	Clear cell carcinoma of human kidney	× [immune cels √]	Human gastric adenocarcinoma	× (immune cells 🗸
luman cerebrum	x	Human skeletal muscle	×	Human astrocytoma	¥ [immune cells ✔]	Human hepatocellular carcinoma	× (immune cells 🗸
Human colon	× (mmune cels √)	Human skin	× (immune cells ✔)	Human bladder cancer	× [immune cells √]	Human lung carcinoma	* (immune cells 🗸
Human endometrium	* (immune cells 🗸 )	Human spleen	1	Human breast carcinoma	× [immune cels √]	Human ovarian carcinoma	* (immune cells 🗸
Human kidney	×	Human stomach	× (immune cels ✓)	Human cervical carcinoma	× [immune cels √]	Human pancreatic carcinoma	* (immune cels 🗸
Human liver	× (Kupffer cells ✔)	Human testis	× (mmune cels ✓)	Human colon carcinoma	¥ [immune cels √]	Human prostatic hyperplasia	x
Human lung	× (mmune cells √)	Human thyroid	× (immune cells ✔)	Human endometrial carcinoma	¥ [mmune cells √]	Human thyroid carcinoma	× (immune cells 🗸
luman mammary gland	× (mmune cels √)	Human tonsil	1				
luman pancreas	× (immune cells √)						

Tissue Microarrays stained for "Anti-CD68 antibody [EPR20545]" using "ab213363" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab213363 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [EPR20545] (ab213363)



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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# Product datasheet

# Anti-FOXP3 antibody [EPR22102-37] ab215206

Recombinant RabMAb

# \*\*\*\*\* 5 Abreviews 15 References 13 Images

# Overview Product name Anti-FOXP3 antibody [EPR22102-37] Description Rabbit monoclonal [EPR22102-37] to FOXP3 Host species Rabbit Specificity ab215206 is not recommended for rat in WB. According to our preliminary WB data, this antibody failed to detect endogenous expression of FOXP3 in the tissues tested in WB, such as thymus. It might work in CD4+CD25+ Treg cells with abundant FOXP3, but we don't have experimental data to support that. **Tested applications** Suitable for: WB, IP, IHC-P, Flow Cyt (Intra) Unsuitable for: ICC/IF or IHC-Fr **Species reactivity** Reacts with: Mouse, Rat, Human Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Positive control** WB: HEK-293T transfected with FOXP3 (WT) expression vector containing a GFP-myc tag, whole cell lysate. Full-length C-MYC/DDK tagged Recombinant Mouse Foxp3 protein. IHC-P: Human thymus and spleen tissues; Mouse thymus and spleen tissues; Rat thymus tissue. Flow Cyt (intra): HEK-293T cell line transfected with a GFP-tagged FOXP3 expression construct. IP: HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate. General notes This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb<sup>®</sup> patents.

Properties	P	ope	rties
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Form

Storage instructions

Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22102-37
Isotype	lgG

# Applications

**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab215206 in the following tested applications.

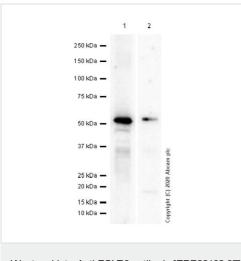
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa). According to our preliminary WB data, this antibody failed to detect endogenous expression of FOXP3 in the tissues tested in WB, such as thymus. It might work in CD4+CD25+ Treg cells with abundant FOXP3, but we don't have experimental data to support that.
IP		1/30.
IHC-P	★★★★ (5)	1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Use at 1/1000 dilution for rat and mouse tissues.
		Recommend to incubate primary antibody at 4C overnight, and use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) as the detection kit.
Flow Cyt (Intra)		1/40.

**Application notes** 

Is unsuitable for ICC/IF or IHC-Fr.

Target	
Function	Probable transcription factor. Plays a critical role in the control of immune response.
Involvement in disease	Defects in FOXP3 are the cause of immunodeficiency polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) [MIM:304790]; also known as X-linked autoimmunity-immunodeficiency syndrome. IPEX is characterized by neonatal onset insulin-dependent diabetes mellitus, infections, secretory diarrhea, trombocytopenia, anemia and eczema. It is usually lethal in infancy.
Sequence similarities	Contains 1 C2H2-type zinc finger. Contains 1 fork-head DNA-binding domain.
Cellular localization	Nucleus.



Western blot - Anti-FOXP3 antibody [EPR22102-37] (ab215206) Lane 1 : Anti-FOXP3 antibody [EPR22102-37] (ab215206) at 1/200 dilution

Lane 2 : Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/1000 dilution (Anti-DDDDK tag, 1:1000 dilution)

All lanes : Full-length C-MYC/DDK tagged Recombinant Mouse Foxp3 protein 10ng

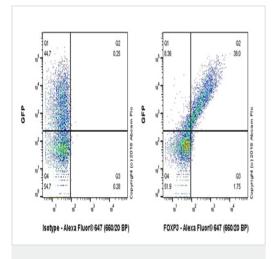
# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

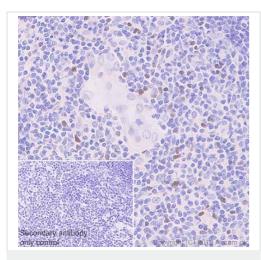
Predicted band size: 47 kDa Observed band size: 50 kDa

## Exposure time: 180 seconds

Blocking buffer and concentration: 5% NFDM /TBST



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206) Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell line transfected with a GFP-tagged FOXP3 expression construct labeling FOXP3 with ab215206 at 1/40 dilution (Right) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left). Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 647) (ab150079), at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)



Immunoprecipitation - Anti-FOXP3 antibody [EPR22102-37] (ab215206) Immunohistochemical analysis of paraffin-embedded human thymus tissue labeling FOXP3 with ab215206 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in human thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

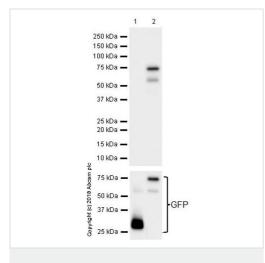
Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent).

FOXP3 was immunoprecipitated from 0.35 mg of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate with ab215206 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab215206 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate 10 µg (Input). Lane 2: ab215206 IP in HEK-293T cells transfected with a GFPtagged FOXP3 expression construct whole cell lysate (+). Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab215206 in HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate (-).

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 seconds.

FOXP3 isoforms have been described in the literature. (PMID: 19568423).



Western blot - Anti-FOXP3 antibody [EPR22102-37] (ab215206) All lanes : Anti-FOXP3 antibody [EPR22102-37] (ab215206) at 1/1000 dilution

Lane 1 : HEK-293T (human embryonic kidney) transfected with an empty vector (vector control), containing a GFP-myc tag, whole cell lysate

Lane 2 : HEK-293T transfected with FOXP3 (WT) expression vector containing a GFP-myc tag, whole cell lysate

Lysates/proteins at 10 µg per lane.

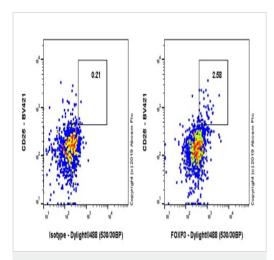
# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 47 kDa Observed band size: 73 kDa

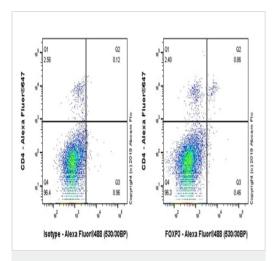
Exposure time: 6 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

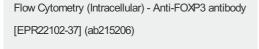


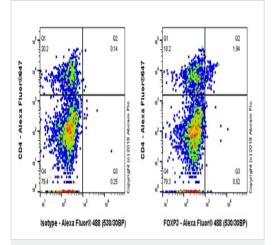
Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206) Human PBMCs were stained with Alexa Fluor<sup>®</sup> 647 conjugated anti-human CD4 and BV421 conjugated anti-human CD25. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear<sup>™</sup> permeabilization buffer, followed by intracellular staining with rabbit IgG (ab172730, Left) or anti-FOXP3 RabMab (Right, 1/40). ab98462, Dylight<sup>®</sup>488-conjugated goat anti-rabbit IgG was used as the secondary at a dilution of 1/2000.

Gating strategy and expression pattern is consistent with literature (PMID: 27330808).

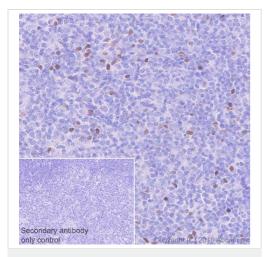


Mouse splenocytes were stained with Alexa Fluor<sup>®</sup> 647 conjugated anti-mouse CD4. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear<sup>™</sup> permeabilisation buffer, followed by intracellular staining with rabbit IgG (ab172730, Left) or anti-FOXP3 RabMab (Right, 1/40). ab150077, Alexa Fluor<sup>®</sup> 488conjugated goat anti-rabbit IgG was used as the secondary at a dilution of 1/2000.





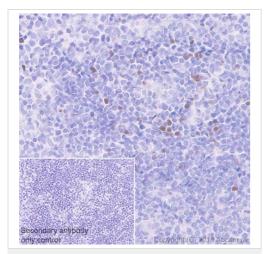
Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206) Rat splenocytes were stained with Alexa Fluor<sup>®</sup> 647 conjugated anti-rat CD4. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear<sup>™</sup> permeabilization buffer, followed by intracellular staining with rabbit IgG (ab172730, Left) or anti-FOXP3 RabMab (Right, 1/40). ab150077, Alexa Fluor<sup>®</sup>488conjugated goat anti-rabbit IgG was used as the secondary at a dilution of 1/2000.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded rat thymus tissue labeling FOXP3 with ab215206 at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in rat thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

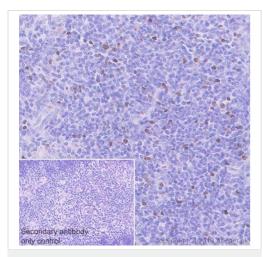
Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling FOXP3 with ab215206 at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in mouse spleen (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

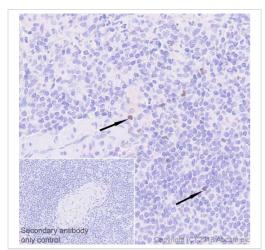
Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded mouse thymus tissue labeling FOXP3 with ab215206 at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in mouse thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)



Anti-FOXP3 antibody [EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling FOXP3 with ab215206 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in human spleen (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using ab208572 (Universal HIER antigen retrieval reagent).

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# Product datasheet

# Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)] ab215838

★★★★★ 2 Abreviews 13 References 4 Images

### Overview

Product name	Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)]
Description	Mouse monoclonal [PAN-CK (Cocktail)] to pan Cytokeratin
Host species	Mouse
Specificity	This antibody cocktail recognizes acidic (Type I or LMW) and basic (Type II or HMW) Cytokeratins, with 67kDa (Cytokeratin1); 64kDa (Cytokeratin3); 59kDa (Cytokeratin4); 58kDa (Cytokeratin5); 56kDa (Cytokeratin6); 55kDa (Cytokeratin7); 52kDa (Cytokeratin8); 56.5kDa (Cytokeratin10); 53kDa (Cytokeratin13); 50kDa (Cytokeratin14); 50kDa (Cytokeratin15); 48kDa (Cytokeratin16); 46kDa (Cytokeratin17); 45kDa (Cytokeratin18) and 40kDa (Cytokeratin19).
Tested applications	Suitable for: IHC-P, Flow Cyt, ICC
Species reactivity	Reacts with: Human
	Predicted to work with: Mouse, Rat, Rabbit, Chicken, Cow, Dog, Monkey 🛛 🔺
Immunogen	Full length native protein (purified) corresponding to Human pan Cytokeratin. Human epidermal keratins.
Positive control	IHC-P: Human basal cell carcinoma and squamous cell carcinoma tissues. ICC and Flow Cyt: HeLa cells.
General notes	lsotype is mouse lgGs, kappa.
	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

# Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.2 Preservative: 0.05% Sodium azide Constituents: 99% PBS, 0.05% BSA
Purity	Protein A/G purified
Purification notes	ab215838 is purified from Bioreactor Concentrate by Protein A/G.
Clonality	Monoclonal
Clone number	PAN-CK (Cocktail)
Isotype	lgG

# Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab215838 in the following tested applications.

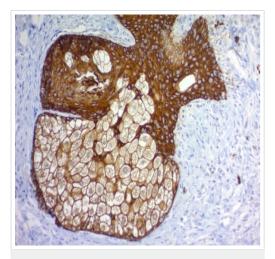
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ІНС-Р	★★☆☆☆ (2)	Use a concentration of 1 - 2 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use 0.5-2 $\mu$ g for 10 <sup>6</sup> cells.
ICC		Use a concentration of 1 - 2 µg/ml.

# Target

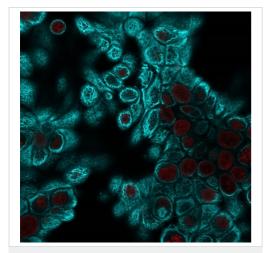
Relevance	Cytokeratins, a group comprising at least 29 different proteins, are characteristic of epithelial and trichocytic cells. Cytokeratins 1, 4, 5, 6, and 8 are members of the type II neutral to basic subfamily. Monoclonal anti cytokeratins are specific markers of epithelial cell differentiation and have been widely used as tools in tumor identification and classification. Monoclonal Anti Pan Cytokeratin is a broadly reactive reagent, which recognizes epitopes present in most human epithelial tissues. It facilitates typing of normal, metaplastic and neoplastic cells. Synergy between the various components results in staining amplification. This enables identification of cells, which would otherwise be stained only marginally. The mixture may aid in the discrimination of carcinomas and nonepithelial tumors such as sarcomas, lymphomas and neural tumors. It is also useful in detecting micrometastases in lymph nodes, bone marrow and other tissues and for determining the origin of poorly differentiated tumors. There are two types of cytokeratins the acidic type I cytokeratins and the basic or neutral type II cytokeratin. Usually the type II cytokeratins are 8kD larger than their type I counterparts.
Cellular localization	Cytoplasmic

# Images

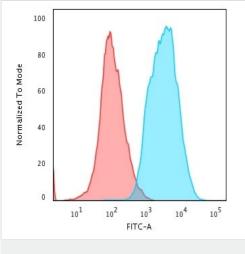


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)] (ab215838)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human basal cell carcinoma tissue labelling pan Cytokeratin with ab215838 at 2 µg/mL for 30 minutes at room temperature. Staining of formalin-fixed tissues requires heating tissue sections in 10mM Tris with 1mM EDTA, pH 9.0, for 45 min at 95°C followed by cooling at room temperature for 20 minutes.



Immunocytochemistry - Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)] (ab215838) Immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling pan Cytokeratin with ab215838 at 2  $\mu$ g/mL followed by Goat anti-Mouse IgG-CF488 (cyan). The nuclear counterstain is NucSpot (red).

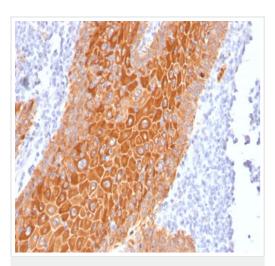


Flow cytometry analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling pan-Cytokeratin with ab215838 at 2µg/10^6 cells followed by Goat Anti-Mouse IgG-CF488 (blue); isotype control (red).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human squamous cell carcinoma tissue

labelling pan Cytokeratin with ab215838 at 2 µg/mL for 30 minutes at room temperature. Staining of formalin-fixed tissues requires heating tissue sections in 10mM Tris with 1mM EDTA, pH 9.0, for 45 min at 95°C followed by cooling at room temperature for 20

Flow Cytometry - Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)] (ab215838)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-pan Cytokeratin antibody [PAN-CK (Cocktail)] (ab215838)

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minutes.

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# Product datasheet

# Anti-CCR7 antibody [EPR23192-57] ab253187

Recombinant RabMAb

# 2 References 9 Images

# Overview **Product name** Anti-CCR7 antibody [EPR23192-57] Description Rabbit monoclonal [EPR23192-57] to CCR7 **Host species** Rabbit **Tested applications** Suitable for: IHC-Fr, IHC-P Unsuitable for: Flow Cyt, IP or WB **Species reactivity** Reacts with: Mouse, Rat, Human Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Immunogen **Positive control** IHC-P: Human tonsil, Human Hodgkin's lymphoma, Mouse spleen and Rat spleen tissues. IHC-Fr: Mouse spleen and Rat spleen tissues. General notes This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb<sup>®</sup> patents.

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23192-57

# Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab253187 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

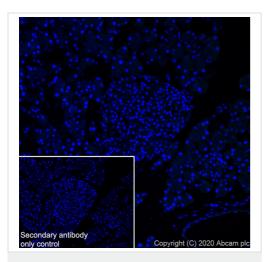
Application	Abreviews	Notes
IHC-Fr		1/100. Perform heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

**Application notes** 

Is unsuitable for Flow Cyt, IP or WB.

Target	
Function	Receptor for the MIP-3-beta chemokine. Probable mediator of EBV effects on B-lymphocytes or of normal lymphocyte functions.
Tissue specificity	Expressed in various lymphoid tissues and activated B- and T-lymphocytes, strongly up-regulated in B-cells infected with Epstein-Barr virus and T-cells infected with herpesvirus 6 or 7.
Sequence similarities	Belongs to the G-protein coupled receptor 1 family.
Cellular localization	Cell membrane.

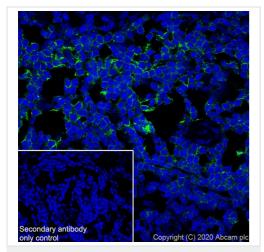
### Images



Immunohistochemistry (Frozen sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse pancreas tissue labeling CCR7 with ab253187 at 1/100 dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution (Green). **Negative control:** No staining on mouse pancreas is observed. The nuclear counterstain was DAPI (Blue).

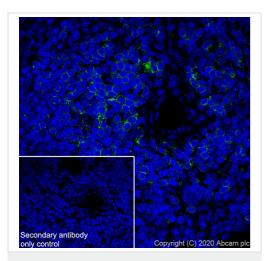
Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution.



Immunohistochemistry (Frozen sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat spleen tissue labeling CCR7 with ab253187 at 1/500 dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution (Green). Cytoplasmic staining on rat spleen is observed. The nuclear counterstain was DAPI (Blue).

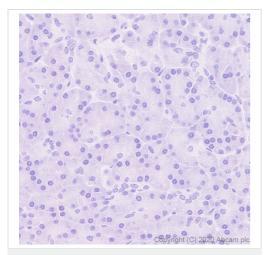
Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution.



Immunohistochemistry (Frozen sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187) Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse spleen tissue labeling CCR7 with ab253187 at 1/500 dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution (Green). Cytoplasmic staining on mouse spleen is observed. The nuclear counterstain was DAPI (Blue).

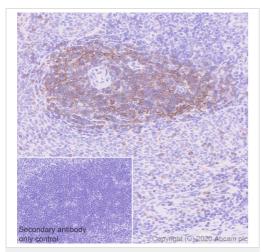
Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 (2 ug/ml) dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).<\p>



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187) Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling CCR7 with ab253187 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). **Negative control:** No staining in human pancreas. The section was incubated with ab253187 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

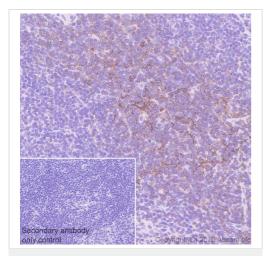
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187) Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling CCR7 with ab253187 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining in rat spleen. The section was incubated with ab253187 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

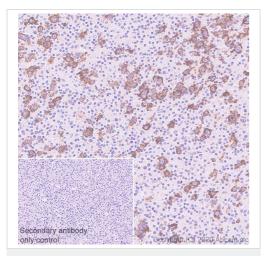


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling CCR7 with ab253187 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining in mouse spleen. The section was incubated with ab253187 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

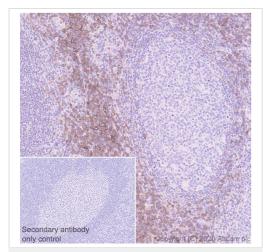


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187)

Immunohistochemical analysis of paraffin-embedded Human Hodgkin's lymphoma tissue labeling CCR7 with ab253187 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on RS cells in human Hodgkin's lymphoma. The section was incubated with ab253187 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CCR7 antibody [EPR23192-57] (ab253187)



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Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling CCR7 with ab253187 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining in human tonsil. The section was incubated with ab253187 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

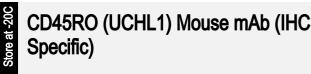
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

# Terms and conditions

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Revision 4







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### For Research Use Only. Not For Use In Diagnostic Procedures.

Applications: IHC-P	<b>Reactivity:</b> H	<b>Sensitivity:</b> Endogenous	Source/Isoty Mouse IgG	<b>/</b>	<b>UniProt ID:</b> P08575	Entrez-Gene Id: 5788
Product Usage Inform	ation			Background		
Application		Dilu	ution	The protein phosphatase (P	TP) receptor CD45 is a type I transmembrane	
Immunohistochemistry (Pa	araffin)	1:4			atase domains and a variable extracellular do tivity of CD45 is a function of the first phospha	
Storage				second phosphatase domain	n (D2) may interact with and stabilize the first acts directly with antigen receptor complex pr	domain, or recruit/bind

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 #36282.

### Specificity / Sensitivity

CD45RO (UCHL1) Mouse mAb (IHC Specific) recognizes endogenous levels of total CD45RO protein. Species Reactivity:

Human

### Source / Purification

Monoclonal antibody is produced by immunizing animals with the IL-2-dependent T cell line, CA1 (5).

kinases involved in the regulation of - and B-cell antigen receipto signaling (1). Specifically, CD45 dephosphorylates Src-family kinases Lck and Fyn at their conserved negative regulatory carboxy-terminal tyrosine residues and upregulates kinase activity. Conversely, studies indicate that CD45 can also inhibit Lck and Fyn by dephosphorylating their positive regulatory autophosphorylation site. CD45 appears to be both a positive and a negative regulator that conducts signals depending on specific stimuli and cell type (1). Human leukocytes including lymphocytes, eosinophils, monocytes, basophils, and neutrophils express CD45, while erythrocytes and platelets are negative for CD45 expression (4). Several isoforms of CD45 are generated through alternative splicing in a cell type-specific and activation state-specific manner. Memory T cells are positive for CD45RO, while naive T cells are negative for CD45RO (5).

- D45RC (5).
  1. Huntington, N.D. and Tarlinton, D.M. (2004) *Immunol Lett* 94, 167-74.
  2. Felberg, J. and Johnson, P. (2000) *Biochem Biophys Res Commun* 271, 292-8.
  3. Kashio, N. et al. (1998) *J Biol Chem* 273, 33856-63.
  4. Wang, Y. and Johnson, P. (2005) *J Biol Chem* 280, 14318-24.
  5. Penninger, J.M. et al. (2001) *Nat Immunol* 2, 389-96.
  6. Smith, S.H. et al. (1986) *Immunology* 58, 63-70.

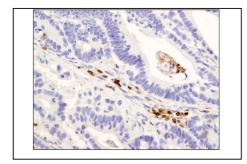
Species reactivity is determined by testing in at least one approved application (e.g., western blot).

#### APPLICATIONS KEY IHC-P: Immunohistochemistry (Paraffin)

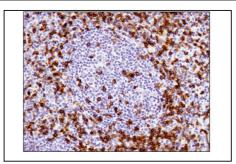
CROSS-REACTIVITY KEY H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus Mi: mink C: chicken Dm: D. melanogaster X: Xenopus Z: zebrafish B: bovine Dg: dog Pg: pig Sc: S. cerevisiae Ce: C. elegans Hr: horse Rab: rabbit All: all species expected

# Revision 4 **#55618** CD45RO (UCHL1) Mouse mAb (IHC Specific)





Immunohistochemical analysis of paraffin-embedded human colon carcinoma using CD45RO (UCHL1) Mouse mAb (IHC Specific).



Immunohistochemical analysis of paraffin-embedded human tonsil using CD45RO (UCHL1) Mouse mAb (IHC Specific).

Revision 4

# **#55618** CD45RO (UCHL1) Mouse mAb (IHC Specific)



# 限制使用

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### Z<sup>中杉金桥</sup> GRIGENE

CD8 抗体试剂 (免疫组织化学) 说明书

#### 【产品名称】

通用名称:CD8抗体试剂(免疫组织化学) 商品名称:抑制/细胞毒T细胞CD8

#### 【包装规格】

产品货号: ZA-0508 ZA-0508UM

工作液:1.5mL/支(1支/袋)、3mL/支(1支/袋)、6mL/支(1支/袋)、18mL/支(1支/ 袋)、25mL/支(1支/袋)。

浓缩液:0.02mL/支(1支/袋)、0.1mL/支(1支/袋)、0.2 mL/支(1支/袋)、1mL/支(1 支/袋),稀释比例见标签。

#### 【预期用途】

本试剂在常规染色(如:HE染色)基础上进行免疫组织化学染色,为医师提供诊断的辅助信息。

#### 【适用范围】

本试剂可用于体外定性检测经 10%中性缓冲福尔马林固定、石蜡包埋人体组织中的 CD8 蛋白。 CD8 分子是存在于抑制/细胞毒 T 细胞表面的 32kDa 的糖蛋白,为 MHC I 类分子的受体,其 胞内部分与 p56lck 酪蛋白激酶相关,调节 TCR/CD3 复合体的功能。可标记抑制/ 细胞毒 T 细胞。 NK 细胞及脾脏窦组织细胞。与 CD4 单抗联合使用,计算 CD4/CD8 比值,对于某些疾病的诊断, 治疗及预后都有一定意义。

#### 【检验原理】

利用抗原抗体特异性结合的原理,本试剂作为一抗,在免疫组织化学染色过程中,特异性结合 组织中的抗原,形成抗原抗体复合物,酶标二抗与该复合物特异性结合,并通过酶促 DAB 显色,使 得组织切片中相应抗原位置出现着色;显微镜检切片,判读结果。

抗原修复采用 EDTA 抗原修复液 pH8.0 高压修复法。

#### 【样本要求】

- 1. 适用样本类型:10%中性缓冲福尔马林固定石蜡组织切片。
- 2. 样本固定:所有组织样本离体后都应尽快经10%中性缓冲福尔马林固定(1小时内),当组织较 大时,应将其每隔5~10mm切开,保证固定液的充分渗透和固定。固定液量应足够,固定时间以6~72小时为宜。
- 3. 包埋及组织切片:组织经石蜡包埋后切片,切片厚度3~5µm,黏附在防脱玻片上,60℃烤片1~2小时。
- 4. 样本的稳定性:为防止抗原丢失,未染色的切片置于室温不宜超过2周,置于2~8°C冰箱不宜超过6周。

#### 【储存条件及有效期】

2~8℃保存,有效期为18个月。

采用泡沫箱加冰袋密封的运输方式,时间不大于7天,开箱温度不超过30℃,产品性能无影响。 使用时应即拿即用,使用后应立即放回冰箱。 生产日期,使用期限或失效日期见标签。

#### 【适用平台】

适用于手工操作和免疫组化染色机染色。

#### 【主要组成成分】

本试剂主要成分为兔抗人 CD8 单克隆抗体(亚型:兔 IgG1; 克隆号: SP16)和缓冲液。 本包装未提供的材料包括:EDTA 抗原修复液 pH8.0、PBS 缓冲液粉末 pH7.3、抗体稀释液、 DAB 染色液(聚合物法)等,试剂信息如下:

编号	材料和试剂 厂家		备注
1	EDTA 抗原修复液 pH8.0	无锡傲锐东源生物科技有限公司	苏锡械备 20160187 号
2	DAB 染色液(聚合物法)	无锡傲锐东源生物科技有限公司	苏锡械备 20160192 号 苏锡械备 20160204 号
3	PBS 缓冲液粉末 pH7.3	无锡傲锐东源生物科技有限公司	苏锡械备 20160190 号
4	抗体稀释液	无锡傲锐东源生物科技有限公司	苏锡械备 20160205 号

#### 【检验方法】

- 手工操作步骤
- 检测所需仪器、设备和耗材
   由热恒温培养箱、光学显微镜、耐高温染色架、高压锅等。
  - 电然但温培齐相、尤子亚似镜、响高温朵巴朵、高压钠;
- 2. 试剂配制

EDTA 抗原修复液 pH8.0、DAB 染色液(聚合物法)、PBS 缓冲液粉末 pH7.3:配制及使用方法 见各自产品说明书。

- 3. 实验步骤
  - 1) 脱蜡和水化

石蜡切片置于新鲜二甲苯中,2缸,浸泡10分钟/缸; 去除多余的液体后,置于无水乙醇中,2缸,浸泡2分钟/缸; 去除多余的液体后,置于95%乙醇中,1缸,浸泡2分钟; 去除多余的液体后,置于75%乙醇中,1缸,浸泡2分钟; 去除多余的液体后,置于75%乙醇中,1缸,浸泡2分钟; 去除多余的液体后,蒸馏水冲洗,置于PBS缓冲液中。

2) 抗原修复

高压锅中,加入 EDTA 抗原修复液 pH8.0,高火预热;待修复液沸腾后将切片置于其中,并 完全浸泡组织,盖好锅盖,扣上压力阀,高火继续加热;待限压阀开始转动喷气后调至中火, 同时开始计时 2.5 分钟;计时结束后离开热源,放气降压后将高压锅移入冷水中冷却;待锅 中液体冷却至室温后取出切片,PBS冲洗缓冲液浸泡清洗2分钟×3次。

1

#### 3) 阻断内源性过氧化物酶

加入 100µL 的内源性过氧化物酶阻断剂(3%H2O2), 室温孵育 10 分钟; 蒸馏水洗 2 次后 甩干液体,用免疫组化笔在距离组织周围 2 ~ 3mm 处画圈。

- 4) 滴加 CD8 抗体试剂或空白对照试剂 滴加 100µL CD8 抗体工作液或空白对照试剂,37℃ 原育 60 分钟或 2~8℃ 原育过夜; PBS 缓冲液浸泡 2 分钟×3 次。
- 5) 滴加酶标羊抗鼠/兔 IgG 聚合物 滴加 100µL 酶标羊抗鼠/兔 IgG 聚合物,37℃孵育 20 分钟;PBS 缓冲液浸泡 2 分钟×3 次。
- 6) 滴加显色剂

加入 100µL 新鲜配制的 DAB 显色剂,室温孵育 5~8 分钟。

7) 复染

自来水冲洗,苏木素染色液室温染色 30~60 秒,自来水冲洗干净,盐酸酒精分化,返蓝。 注意:需要控制苏木素染色液的染色强度。颜色过浅或过深都会干扰显色结果的观察。

- 8) 脱水、透明、封片。
- 9) 结果判读

在光学显微镜下对染色后切片进行观察和结果判读。

• 仪器操作步骤

进入软件输入病例信息:选择需要染色的抗体指标及相应染色程序,打印标签。将标签粘贴至 相应切片后,将切片加载到染色机上。将染色所需的试剂加载到染色机相应区域。启动染色程序, 进行染色。

● 质量控制

每一批次检测样本均应同时设立阳性/阴性对照及空白对照。

阳性对照组织可使用扁桃体。通常阳性对照组织中,存在大量未染色细胞,可作为阴性质控位 点。阴性对照组织用于监控试剂及操作过程是否正确,是否存在非特异性染色。如果阴性对照组织 染色结果为阳性或大量背景着色,则待测样本的检测结果应视为无效。

空白对照试剂一般可用抗体稀释液代替。通常用于替代一抗检测质控组织,用于判断是否存在 非特异性染色或污染。如果空白对照试剂质控染色结果为阳性或大量背景着色,则实验标本的检测 结果应视为无效。

应有 HE (苏木精-伊红染色法)染色结果作为对照。

#### 【阳性判断值】

阳性:检测组织目标细胞可观察到棕黄色细胞膜染色。 明性:检测组织目标细胞末观察到棕黄色细胞膜染色。

#### 【检测方法的局限性】

- 免疫组织化学检测是一种需要通过多个检测步骤完成的诊断过程。在试剂的选择、取材、固定、 处理、切片的制备、染色结果的解释上需要进行专门的培训。
- 任何阳性或阴性结果的解读,应由病理科医生结合病理形态学、临床表现及其它检测方法进行, 不作为单独的诊断指标。
- 3. 复染过度或不足都可能影响结果的判读。

- 不恰当的染色前组织的处理过程直接影响染色效果,造成假阳性、抗体定位不准确或假阴性结果。
   结果不一致可能是由样本固定和包埋方法不同或组织样本内固有差异造成的。
- 5. 阴性结果表示未检出抗原,不一定表示样本中无该抗原存在。待测抗原编码基因变异、抗原低表达、抗原修复不当或孵育时间不足等,都会造成抗原无法检出。

#### 【产品性能指标】

1. 批内重复性

同一组织来源的组织片染色的强度和定位无明显差异。

 批间重复性 不同批号试剂对同一组织来源的组织片染色的强度和定位无明显差异。

#### 【注意事项】

- 1. 本试剂仅用于体外诊断,不做其它用途。
- 2. 本试剂需专业人员使用。
- 3. 检测结束后,试剂和样本的处理应符合相关要求。
- 4. 试剂使用中,应有适当的防护措施,以避免试剂同皮肤和眼睛接触。
- 5. 不宜使用其它生产商的配套试剂与本试剂进行免疫组化染色实验。

#### 【参考文献】

纪小龙,张雷主编.诊断免疫组织化学.第3版北京:人民军医出版社,2011.
 保如荣马大烈.戴益名主编.免疫组织化学实验技术及应用.第1版.北京.化学工业出版社,2006.

#### 【基本信息】

#### 备案人/生产企业名称:无锡傲锐东源生物科技有限公司

住 所:无锡市滨湖区马山梅梁路 168 号
 电 话:0510-81830200 传 真:0510-81830201
 售后服务单位名称:北京中杉金桥生物技术有限公司
 电 话:010-63470385 400-810-9781 技术
 生产地址:无锡市滨湖区马山梅梁路 168 号
 生产备案凭证编号:苏锡械生产备 20150011 号

技术支持:010-63634171

#### 【医疗器械备案凭证编号/产品技术要求编号】

苏锡械备 20180306 号

#### 【说明书核准日期及修改日期】

核准日期:2018.04.04 修改日期:2020.08.03



# HRP 标记二抗抗体-鼠兔混合型说明书

# 【背景】

本产品为辣根过氧化物酶(HRP)聚合物标记山羊抗小鼠和兔混合型 lgG,主要用于免疫 组织化学染色(IHC)等的二抗。本产品不含抗生物素蛋白(avidin)或生物素(biotin), 所以可完全消除内源性生物素引起的非特异性染色。本产品具有特异性强、灵敏度高等特 点。

# 【货号】

10013001050

# 【规格】

50ml

# 【宿主】

山羊

# 【Ig 类型】

lgG

# 【种属反应性】

小鼠、兔

# 【适用组织】

石蜡切片

# 【染色方法】

参见佰诺全景多重荧光免疫组化试剂盒说明书。

# 【IHC 建议稀释比】

直接使用。禁止稀释后使用。

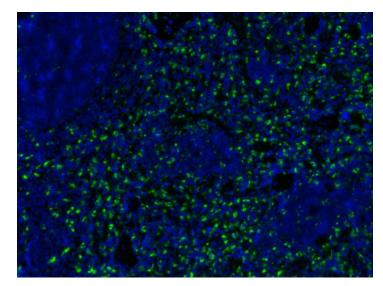
# 【保存条件及效期】

2~8℃保存,有效期12个月,开封后有效期3个月,请尽快使用。生产日期和失效日期 见包装标签。



产品说明书

【应用举例】



使用 CD68 兔单克隆抗体 (1:5000 稀释) 在大鼠脾脏石蜡切片上进行 PPD520 免疫荧光染色, 结果以免疫组化的形式展现。

# 【注意事项】

- 1. 本试剂只用于免疫组化,不做其他用途。
- 2. 本试剂仅限于专业人员使用。
- 3. 应采用适当的防护措施,以避免试剂同皮肤和眼睛接触。
- 4. 超过有效期的试剂活性可能降低,因此不得使用超过有效期的试剂。
- 5. 为了防止可能出现的假阴性、假阳性结果,实验过程中需有阳性对照及阴性对照同时进行。
- 6. 使用本试剂所产生的各种废弃物都应按照《医疗废物管理条例》进行处理。

# 【基本信息】

生产企业: 佰诺全景生物技术(北京)有限公司

- 联系电话: 13681138480
- 电子邮箱: service@panovue.cn

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