abcam

Product datasheet

Anti-CD68 antibody [EPR20545] ab213363

Recombinant RabMAb

★★★★★ 3 Abreviews 29 References 10 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. ICC/IF: THP-1 and U937 cells.
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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® 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, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20545
Isotype	IgG

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		1/1000. 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.

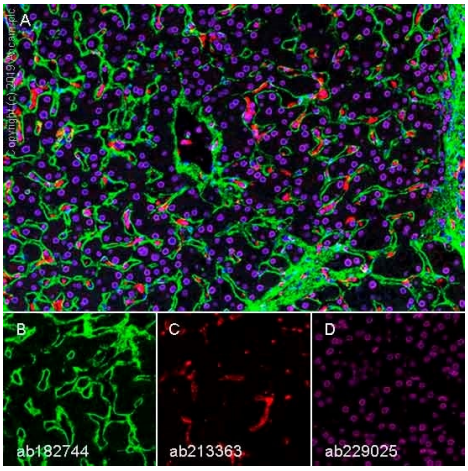
Tissue specificity Highly expressed by blood monocytes and tissue macrophages. Also expressed in lymphocytes, fibroblasts and endothelial cells. Expressed in many tumor cell lines which could allow them to attach to selectins on vascular endothelium, facilitating their dissemination to secondary sites.

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 (Formalin/PFA-fixed paraffin-embedded 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.

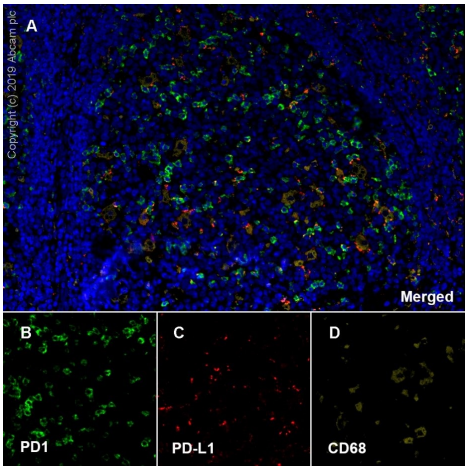
Panel D: Anti-Lamin B1 (magenta) stained on nuclear envelope.

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

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™520), anti-PD-L1 (red, Opal™570) and anti- CD68 (yellow, Opal™690).

Panel B: Anti- PD1 stained on antigen-stimulated T cells.

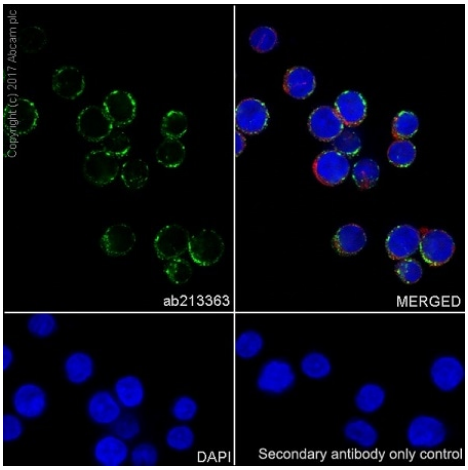
Panel C: anti- PD-L1 stained on cells involved in T cell inhibition

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™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

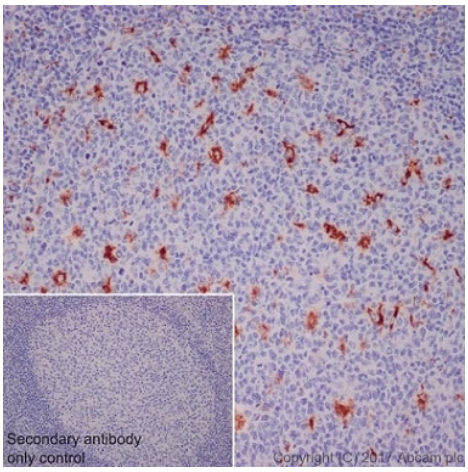


Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] (ab213363)

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® 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® 594) (ab195889) (red) at 1/200 dilution.

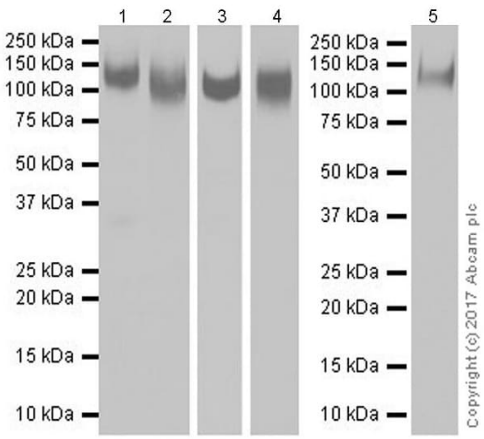
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.



Western blot - Anti-CD68 antibody [EPR20545] (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 µg
Lane 2 : Human tonsil lysate at 20 µg
Lane 3 : Human fetal spleen lysate at 20 µg
Lane 4 : THP-1 (human monocytic leukemia cell line) whole cell lysate at 10 µg
Lane 5 : U937 (human histiocytic lymphoma cell line) whole cell lysate at 10 µg

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

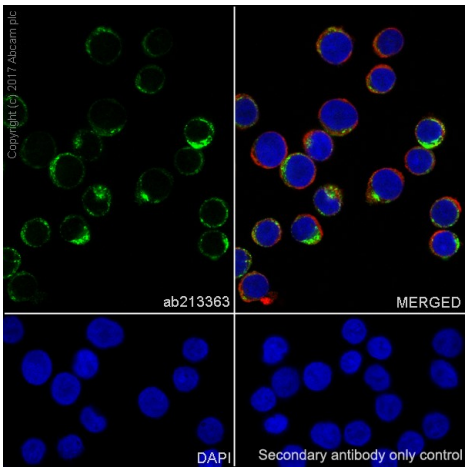
Developed using the ECL technique.

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).

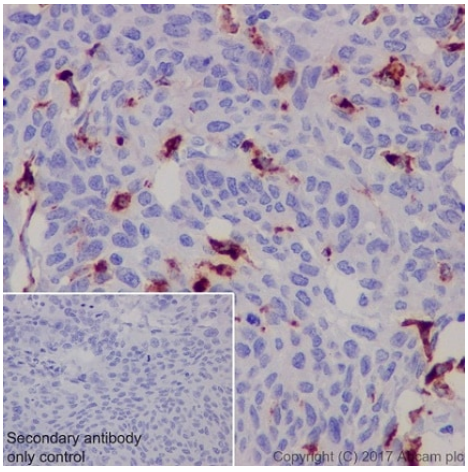


Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] (ab213363)

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® 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® 594) (ab195889) (red) at 1/200 dilution.

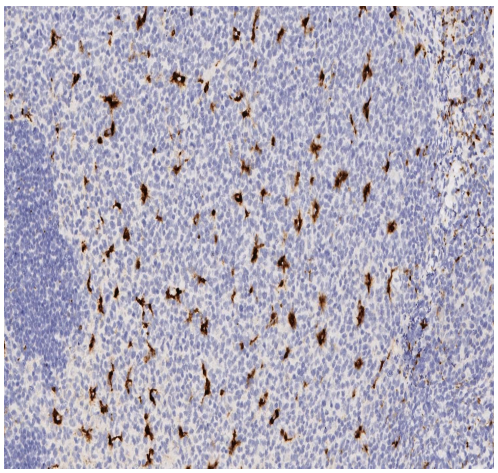
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



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

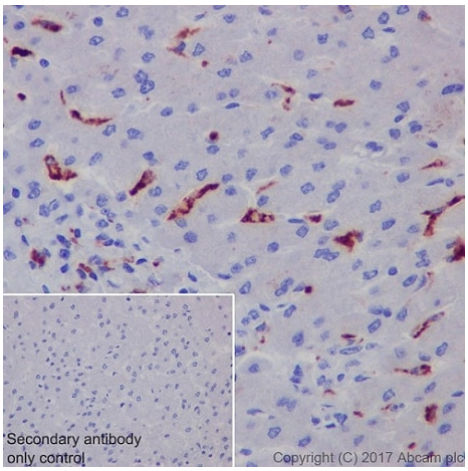
Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue labeling CD68 with ab213363 at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on macrophages of human cervical carcinoma is observed (PMID: 12118106). Counter stained with 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.



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

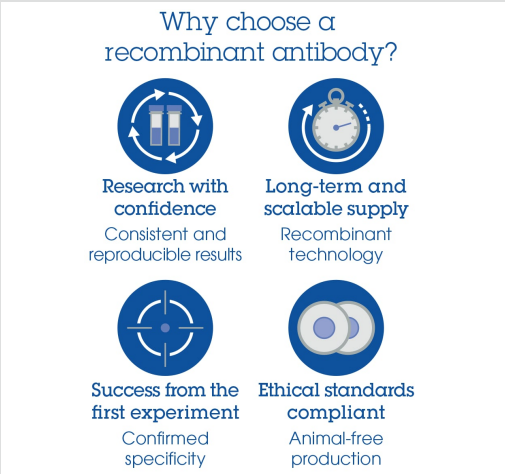
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD68 with ab213363 at 1/5000 dilution. No blocking step performed. Anti-Rabbit HRP polymer was used as the secondary detection system. Heat-mediated antigen retrieval was performed using EDTA based pH 9.0 buffer.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.



Anti-CD68 antibody [EPR20545] (ab213363)

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