abcam

ab283654

Anti-CD68 antibody [EPR23917-164]

Anti-CD68 antibody [EPR23917-164] (ab283654) is a rabbit monoclonal antibody detecting CD68 in **Western Blot, Flow Cytometry (Intra), IHC-P, IHC-Fr, ICC/IF, mIHC**. Suitable for **Mouse, Rat**.

- KO validated for confirmed specificity
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 150 publications

Chicken version of this clone also available as ab318303. Same clone, same staining pattern, different species antibody backbone.
Learn more about chimeric antibodies

Lab Essentials | Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR23917-164
Purification technique	Affinity purification Protein A
Specificity	Mouse unsuitable for IHC-Fr application
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Mouse
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Rat

Dilution info	1/100
Notes	Mouse unsuitable for IHC-Fr application

Not recommended

Species	Mouse
Dilution info	-
Notes	Mouse unsuitable for IHC-Fr application

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human
Dilution info	-
Notes	-

Target data

See full target information Cd68

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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot

Storage information	Avoid freeze / thaw cycle

Notes

Anti-CD68 antibody [EPR23917-164] (ab283654) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-Fr, IHC-P, mIHC, and WB.

Abcam's high quality manufacturing and validation processes ensure Anti-CD68 antibody [EPR23917-164] (ab283654) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-CD68 antibody [EPR23917-164] (ab283654) has been confirmed by Western Blot testing in CD68 knockout RAW 264.7 cells (cell lysate ab280106).

Anti-CD68 antibody [EPR23917-164] (ab283654) specifically detects CD68 (UniProt ID: P31996; Molecular weight: 33kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EPR23917-164 - ab283667.

Antibody clone EPR23917-164 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 647, Alexa Fluor® 594, APC, Alexa Fluor® 488 (ab305214, ab312903, ab317758, ab319086).

CD68 levels is implicated in autoimmune conditions like rheumatoid arthritis, where macrophages contribute to joint inflammation and damage.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD68 is a glycoprotein that functions as a scavenger receptor. It plays an important role in the regulation of cellular debris and apoptotic cell clearance. Researchers often refer to it as KP1 or macrosialin in the literature. CD68 with a molecular weight of approximately 110 kDa is highly expressed in macrophages and monocytes. The mucin-like structure of CD68 allows it to be heavily glycosylated which aids in its function. Researchers detect CD68 in tissues through

	methods like immunohistochemistry (IHC) immunofluorescence and CD68 staining and it is widely used as a macrophage marker in research.
Biological function summary	CD68 facilitates functions associated with the innate immune response. It serves as an important part of macrophages which contribute to immune surveillance and tissue homeostasis. Though it primarily operates on its own in some contexts CD68 might assist in forming complexes with other receptors to modulate phagocytic activity. This target is significant in atherosclerotic plaque stability as macrophages engulf lipids and cell debris through processes facilitated by CD68.
Pathways	CD68 is involved in pathways connected to inflammation and phagocytosis. The CD68 protein works closely with other scavenger receptors like CD36 to mediate uptake of oxidized low-density lipoproteins (oxLDL) in the lipid metabolism pathway. Additionally CD68 engagement can influence the toll-like receptor (TLR) signaling pathways thereby linking innate immunity and inflammatory responses critical for host defense and disease progression.
Associated diseases and disorders	CD68 is associated with atherosclerosis and multiple sclerosis. In atherosclerosis its role becomes evident as it accumulates in macrophages within the plaques making it a marker of disease severity. In multiple sclerosis CD68 expression in macrophages and microglia indicates active demyelination and inflammation. It also relates to proteins such as CD36 in atherosclerosis where both mediate the uptake of modified LDL contributing to foam cell formation in plaques.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

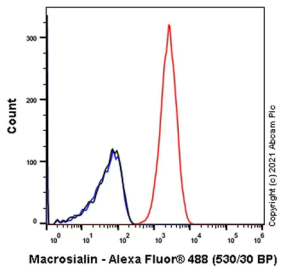
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

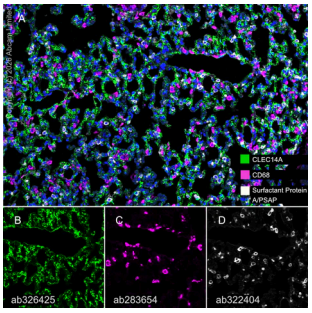
26 product images



Flow Cytometry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Flow Cytometry staining using rabbit Anti-CD68 antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling CD68 with ab283654 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat F(ab')2 Anti-Rabbit IgG(DyLight® 488, ab98507) at 1/500 dilution was used as the secondary antibody.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of Mouse lung using rabbit Anti-CD68 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining CLEC14A with ab326425 at a 1/500 dilution (0.994 µg/ml), ab283654 anti-CD68 at a 1/100 dilution (4.43 µg/ml) and ab322404 anti-Surfactant Protein A/PSAP at a 1/5000 dilution (0.10 µg/ml).
Panel A: merged staining of anti-CLEC14A (green; Opal^{TM}570), anti-CD68 (magenta; Opal^{TM}690) and anti-Surfactant Protein A/PSAP (gray; Opal^{TM}520) on mouse lung.

Panel B: anti-CLEC14A staining endothelium in mouse lung.

Panel C: anti-CD68 staining macrophages in mouse lung.

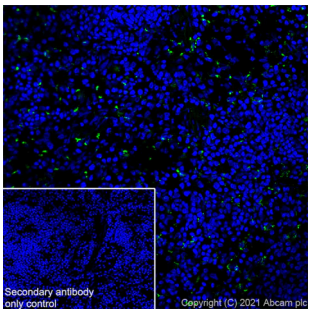
Panel D: anti-Surfactant Protein A/PSAP staining type II alveolar epithelial cells in mouse lung.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab326425, ab283654 and ab322404 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^{TM} 4-color kit using Opal Polymer HRP Ms + Rb secondary reagent. Image acquisition was performed with Leica SP8 confocal microscope.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

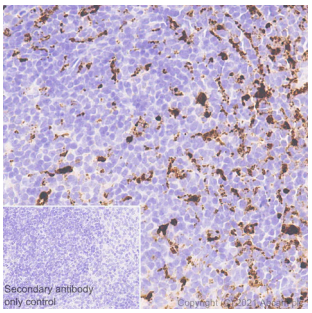


Immunohistochemistry (Frozen sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Frozen sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat spleen (fresh) tissue labeling CD68 with ab283654 at 1/100 (4.66 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution (Green). Positive staining on macrophage of rat spleen is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

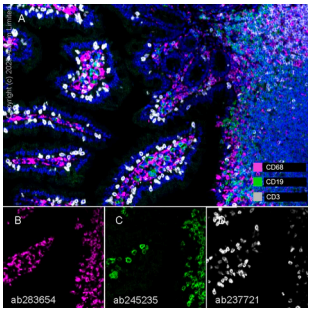


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on mouse spleen. The section was incubated with ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



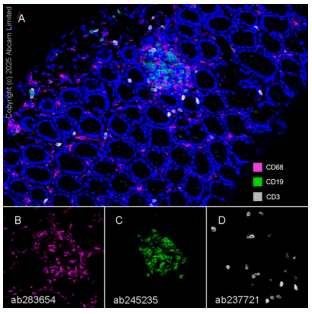
Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse small intestine tissue staining CD68 with ab283654 at a 1/100 dilution, ab245235 anti-CD19 used at 1/1000 dilution and ab237721 anti-CD3 used at a 1/2000 dilution. Opal Polymer HRP Ms + Rb 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 solution2) for 20 mins.

Panel A: merged staining of anti-CD68 (magenta; Opal™520), anti-CD19 (green; Opal™690) and anti-CD3 (grey; Opal™570) on mouse small intestine.
Panel B: anti-CD68 staining macrophages in mouse small intestine.
Panel C: anti-CD19 staining B lymphocytes in mouse small intestine.
Panel D: anti-CD3 staining T lymphocytes in mouse small intestine.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab283654, ab245235 and ab237721 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.



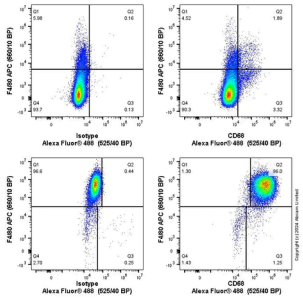
Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse colon tissue staining CD68 with ab283654 at a 1/100 dilution, ab245235 anti-CD19 used at 1/1000 dilution and ab237721 anti-CD3 used at a 1/2000 dilution. Opal Polymer HRP Ms + Rb 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 solution2) for 20 mins.

Panel A: merged staining of anti-CD68 (magenta; Opal™520), anti-CD19 (green; Opal™690) and anti-CD3 (grey; Opal™570) on mouse colon.
Panel B: anti-CD68 staining macrophages in mouse colon.
Panel C: anti-CD19 staining B lymphocytes in mouse colon.
Panel D: anti-CD3 staining T lymphocytes in mouse colon.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab283654, ab245235 and ab237721 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.



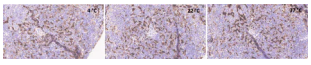
Flow Cytometry (Intracellular) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Flow Cytometry (Intracellular) staining of Mouse Bone Marrow Cell-derived Macrophages using rabbit Anti-CD68 antibody

Flow cytometry staining of C57BL/6 mouse splenocytes (top) or C57BL/6 Mouse Bone Marrow Cell-derived Macrophages (bottom) with ab283654 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min. Cells were incubated for 30min on ice in 1x PBS containing 10µg/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab283654 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6in 100 µl at 1.0 µg/ml (1/2212)) for 30min at 22°C. The cells were simultaneously stained with F4/80.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.

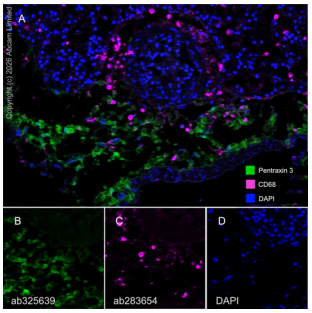


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse spleen tissue using rabbit Anti-CD68 antibody

The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Paraffin embedded mouse spleen tissue slides were dewaxed, followed by heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, Epitope Retrieval ER2 Solution) for 20 mins. Endogenous peroxidase activity was blocked by Refine Detection Kit Peroxide Block for 10 mins. The sections were then incubated with ab283654 (1/100) at room temperature for 30 mins, followed by a ready to use LeicaDS9800 (BOND Polymer Refine Detection Kit). The sections were counterstained with Hematoxylin. The images were taken with a Leica Aperio AT2.

ab283654 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in IHC-P. The image shows the staining intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’s transition from cold⊠chain to ambient shipping.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of Mouse lung treated with LPS (1ug/ml 16h)+BFA (1ug/ml 16h) (+) ex vivo using rabbit Anti-CD68 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung treated with lps (1ug/ml 16h)+bfa (1ug/ml 16h) (+) ex vivo tissue staining Pentraxin 3/PTX3 with ab325639 at a 1:100 (5.07 µg/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.15 µg/ml) dilution.

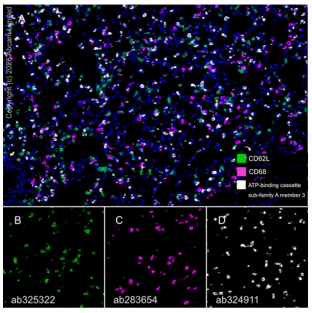
Panel A: merged staining of anti-Pentraxin 3 (green; Opal™520), anti-E CD68 (magenta; Opal™570) on mouse lung treated with LPS (1ug/ml 16h)+BFA (1ug/ml 16h) ex vivo.
Panel B: anti-Pentraxin 3 staining stroma cells in mouse lung treated with LPS (1ug/ml 16h)+BFA (1ug/ml 16h) ex vivo.
Panel C: anti-CD68 staining macrophages in mouse lung treated with LPS (1ug/ml 16h)+BFA (1ug/ml 16h) ex vivo.
Panel D: Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab325639 and ab283654 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.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of Mouse lung using rabbit Anti-CD68 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining CD62L with ab325322 at a 1/1000 (0.509 µg/ml) dilution, ab283654 anti-CD68 used at a 1/100 (4.43 µg/ml) dilution and ab324911 anti-ATP-binding cassette sub-family A member 3 used at a 1/2000 (0.253 µg/ml) dilution.
Panel A: anti-CD62L (green; Opal™520), anti-CD68 (magenta; Opal™690), anti-ATP-binding cassette sub-family A member 3 (gray; Opal™570) on mouse lung.

Panel B: anti-CD62L staining immune cells in mouse lung.

Panel C: anti-CD68 staining macrophages in mouse lung.

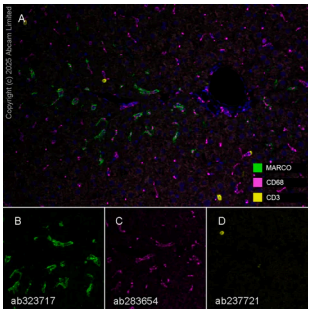
Panel D: anti-ATP-binding cassette sub-family A member 3 staining type ☒ pneumocytes in mouse lung.

Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab325322, ab283654 and ab324911 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.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of rat liver tissue using rabbit Anti-CD68 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded rat liver tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

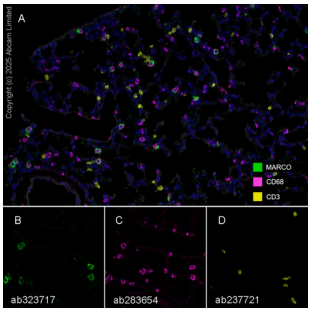
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD68 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on rat liver.
Panel B: anti-MARCO staining macrophages in rat liver.
Panel C: ant-CD68 staining macrophages in rat liver.
Panel D: ant-CD3 staining T lymphocytes in rat liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab283654 and ab237721 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.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of mouse lung tissue using rabbit Anti-CD68 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse lung tissue staining MARCO with ab323717 at a 1:200 (2.49 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.26 ug/ml) dilution.

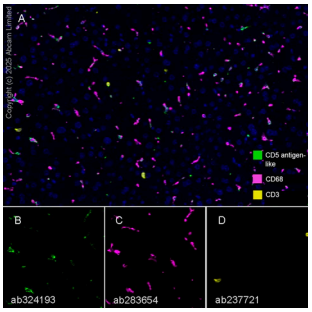
Panel A: merged staining of anti-MARCO (green; Opal™520), anti-CD68 (magenta; Opal™690) and anti-CD3 (yellow; Opal™570) on mouse lung.
Panel B: anti-MARCO staining macrophages in mouse lung.
Panel C: ant-CD68 staining macrophages in mouse lung.
Panel D: ant-CD3 staining T lymphocytes in mouse lung.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab323717, ab283654 and ab237721 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.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Multiplex immunohistochemistry staining of Mouse liver using rabbit Anti-CD68 antibody

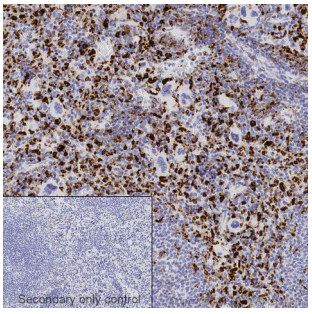
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded mouse liver tissue staining CD5L/CT-2 with ab324193 at a 1:500 (0.958 ug/ml) dilution, ab283654 anti-CD68 used at 1:100 (4.43 ug/ml) dilution and ab237721 anti-CD3 used at a 1:2000 (0.25 ug/ml) dilution.

Panel A: merged staining of anti-CD5 antigen-like (green; Opal™520), anti-CD68 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on mouse liver.
Panel B: anti-CD5 antigen-like staining macrophages in mouse liver.
Panel C: anti-CD68 staining macrophages in mouse liver.
Panel D: anti-CD3 staining T lymphocytes in mouse liver.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324193, ab283654 and ab237721 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



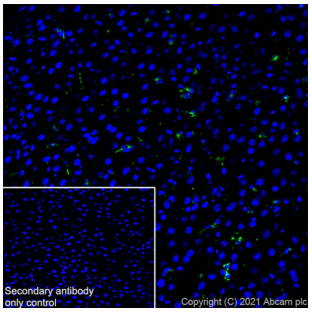
Immunohistochemistry - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry staining of Mouse spleen using rabbit Anti-CD68 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded mouse spleen labelling CD68 with ab283654 at a concentration of 0.1µ/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab283654 anti-CD68 antibody [EPR23917-164] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

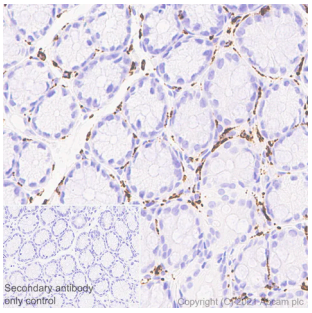
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Immunohistochemistry (Frozen sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Frozen sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat liver (fresh) tissue labeling CD68 with ab283654 at 1/100 (4.66 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution (Green). Positive staining on Kupffer cells of rat liver is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

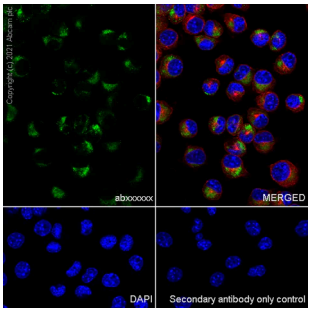


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on immune cells in mouse colon. The section was incubated with ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

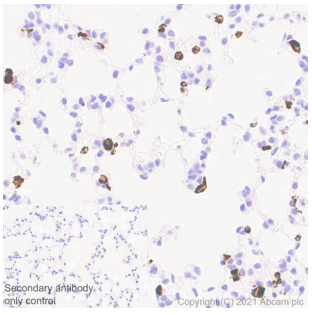
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD68 antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized J774A.1 cells labelling CD68 with ab283654 at 1/50 (9.32 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing cytoplasmic staining in J774A.1 cell line. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

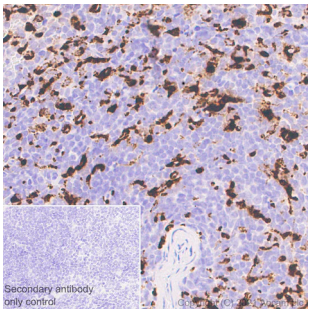
CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on macrophages in rat lung. The section was incubated with

ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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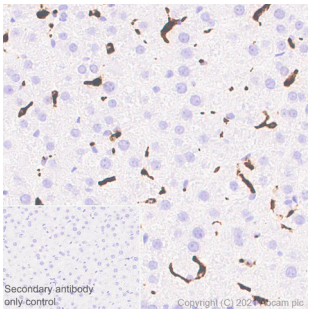
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on rat spleen. The section was incubated with ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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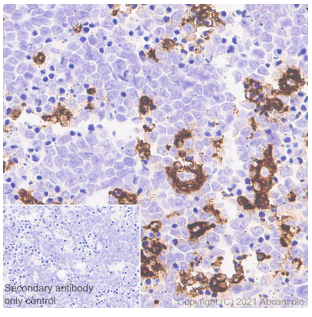
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Rat liver tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on Kupffer cells in rat liver. The section was incubated with ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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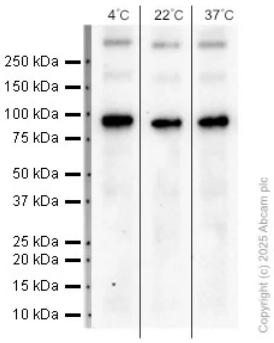
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD68 antibody

Immunohistochemical analysis of paraffin-embedded Mouse large B cell lymphoma tissue labelling CD68 with ab283654 at 1/100 (4.66 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on mouse large B cell lymphoma. The section was incubated with ab283654 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 LeicaDS9800 (Bond™ Polymer Refine Detection) .

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Western blot staining of Mouse spleen lysate using rabbit Anti-CD68 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of mouse spleen lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab283654 (1/5000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/50,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab283654 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

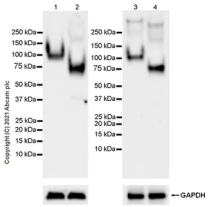
All lanes:
Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654) at 1/5000 dilution

All lanes:
Mouse spleen lysate at 15 µg with NDFM/TBST

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Exposure time: 180s



Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Western blot staining using rabbit Anti-CD68 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST
Macrosialin (CD68) is a glycoprotein and can be de-glycosylated by PNGase F. The molecular mass observed is consistent with the literature (PMID: 7680921)

Exposure time: Lane 1-2: 7.75 seconds; Lane 3-4: 48 seconds

All lanes:
Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654) at 1/1000 dilution

Lane 1:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate at 15 µg

Lane 2:
RAW264.7 treated with PNGase F whole cell lysate at 15 µg

Lane 3:

Mouse spleen tissue lysate at 15 µg

Lane 4:
Mouse spleen treated with PNGase F tissue lysate at 15 µg

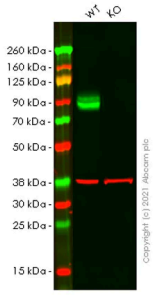
Secondary

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

Predicted band size: 37 kDa

Observed band size: 100 kDa

Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654)



CD68 Western blot staining using rabbit Anti-CD68 antibody

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.
Lanes 1-2: Merged signal (red and green). Green - ab283654 observed at 100 kDa. Red - loading control ab8245 observed at 36 kDa.
ab283654 Anti-CD68 antibody [EPR23917-164] was shown to specifically react with CD68 in wild-type RAW264.7 cells. Loss of signal was observed when knockout cell line (knockout cell lysate - ab280106) was used. Wild-type and CD68 knockout samples were subjected to SDS-PAGE. ab283654 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4☒ overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654) at 1/1000 dilution

Lane 1:
Wild-type RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

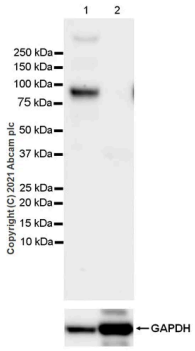
Lane 2:
Western blot - Mouse CD68 knockout RAW 264.7 cell lysate (ab280106) at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/10000 dilution

Predicted band size: 37 kDa

Observed band size: 100 kDa



Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Western blot staining using rabbit Anti-CD68 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST
Negative control: mouse skeletal muscle (PMID: 28091823).

Exposure time: 37 seconds

All lanes:
Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654) at 1/1000 dilution

Lane 1:
Mouse spleen tissue lysate at 20 µg

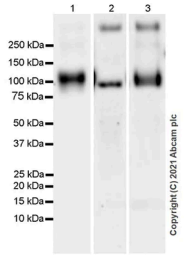
Lane 2:
Mouse skeletal muscle tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 37 kDa

Observed band size: 100 kDa



Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654)

CD68 Western blot staining using rabbit Anti-CD68 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST
Exposure time: Lane 1: 7.75 seconds; Lane 2: 125 seconds; Lane 3: 92 seconds

All lanes:
Western blot - Anti-CD68 antibody [EPR23917-164] (ab283654) at 1/1000 dilution

Lane 1:
J774A.1 (mouse reticum cell sarcoma monocyte macrophage) whole cell lysate 20 at 20 µg

Lane 2:
Rat liver tissue lysate at 20 µg

Lane 3:
Rat spleen tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 37 kDa

Observed band size: 100 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.