abcam

Product datasheet

Anti-CD68 antibody [EPR23917-164] - BSA and Azide free ab283667

KO VALIDATED Recombinant RabMAb

1 Abreviews 15 Images

Overview

Product name	Anti-CD68 antibody [EPR23917-164] - BSA and Azide free
Description	Rabbit monoclonal [EPR23917-164] to CD68 - BSA and Azide free
Host species	Rabbit
Specificity	Mouse unsuitable for IHC-Fr application
Tested applications	**Suitable for:** IHC-P, ICC/IF, WB, IHC-Fr, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Mouse spleen, RAW264.7, Mouse spleen, J774A.1, Rat liver and Rat spleen lysates. IHC-P: Mouse spleen, Mouse colon, Mouse large B lymphoma, Rat spleen, Rat lung and Rat liver tissues. IHC-Fr: and Rat liver, Rat spleen tissues. ICC/IF: J774A.1 cells. Flow Cyt: RAW 264.7 cell.
General notes	ab283667 is the carrier-free version of **ab283654**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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.
Storage buffer	pH: 7.2 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23917-164
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab283667 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	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 37 kDa.
IHC-Fr		Use at an assay dependent concentration. Mouse unsuitable for IHC-Fr application
Flow Cyt (Intra)		Use at an assay dependent concentration.

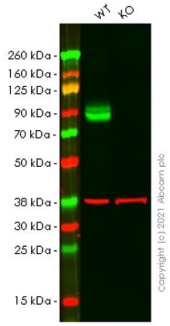
Application notes Is unsuitable for IP.

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



Western blot - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

All lanes : 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
Lane 2 : Mouse CD68 knockout RAW 264.7 cell lysate (**ab280106**)

Lysates/proteins at 20 µg per lane.

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

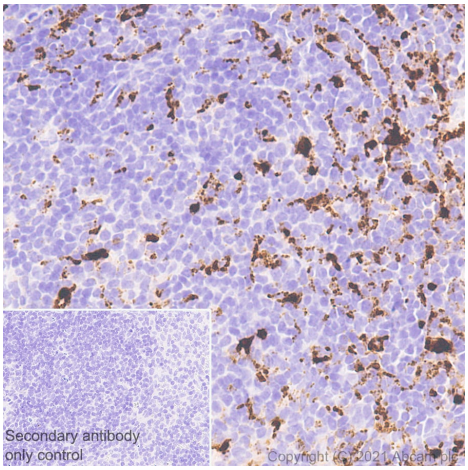
Predicted band size: 37 kDa
Observed band size: 100 kDa

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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.



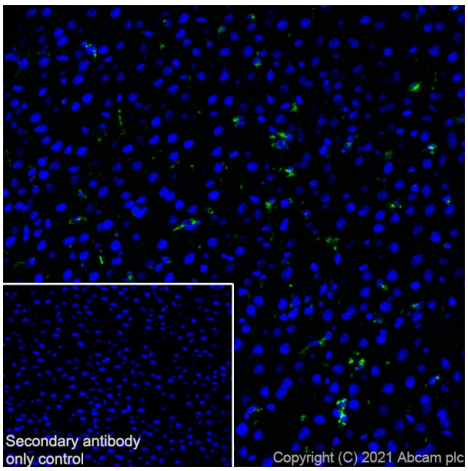
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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

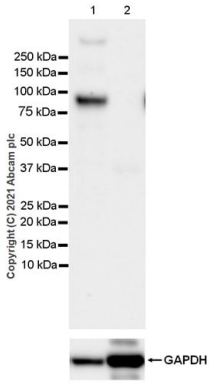


Immunohistochemistry (Frozen sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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.



Western blot - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

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

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Mouse skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

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

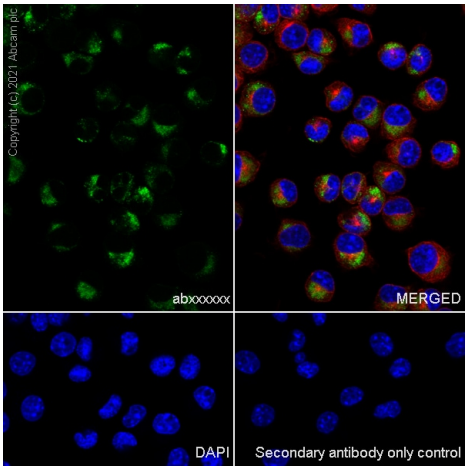
Predicted band size: 37 kDa
Observed band size: 100 kDa

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Negative control: mouse skeletal muscle (PMID: 28091823).

Exposure time: 37 seconds

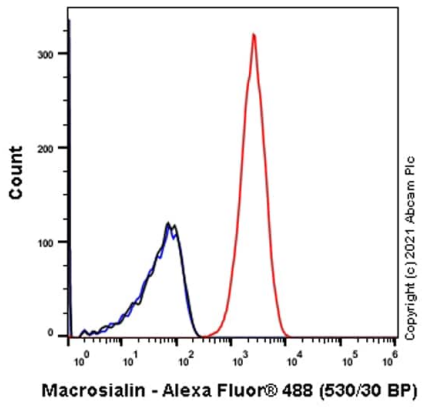


Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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).

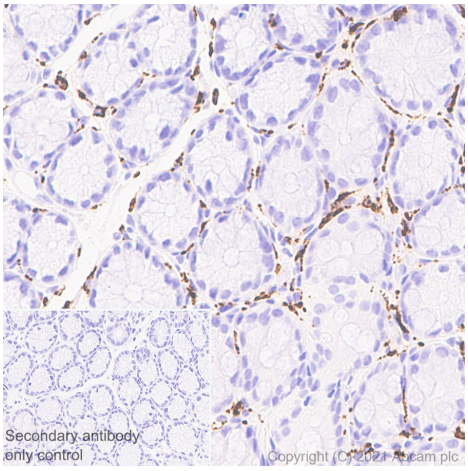
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow Cytometry - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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.



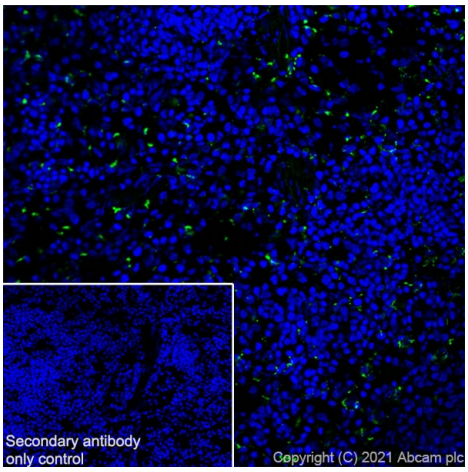
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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

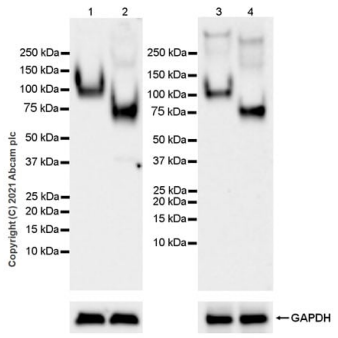


Immunohistochemistry (Frozen sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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.



Western blot - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

All lanes : 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
Lane 2 : RAW264.7 treated with PNGase F whole cell lysate
Lane 3 : Mouse spleen tissue lysate
Lane 4 : Mouse spleen treated with PNGase F tissue lysate

Lysates/proteins at 15 µg per lane.

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

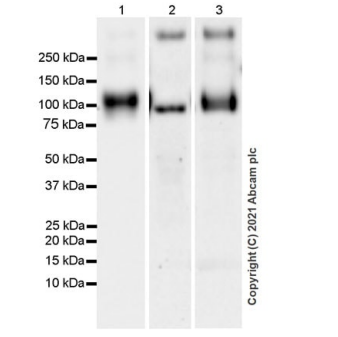
Predicted band size: 37 kDa
Observed band size: 100 kDa

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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



Western blot - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

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

Lane 1 : J774A.1 (mouse reticum cell sarcoma monocyte macrophage) whole cell lysate 20
Lane 2 : Rat liver tissue lysate
Lane 3 : Rat spleen tissue lysate

Lysates/proteins at 20 µg per lane.

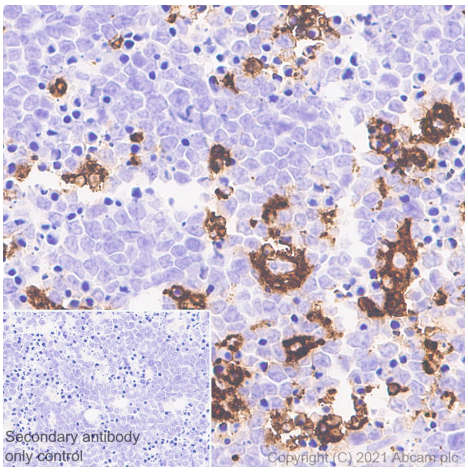
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/50000 dilution

Predicted band size: 37 kDa
Observed band size: 100 kDa

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: Lane 1: 7.75 seconds; Lane 2: 125 seconds; Lane 3: 92 seconds



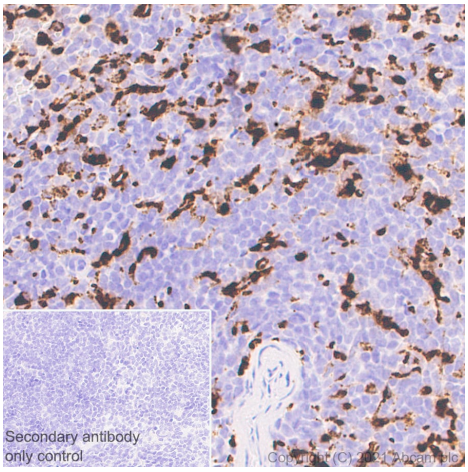
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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



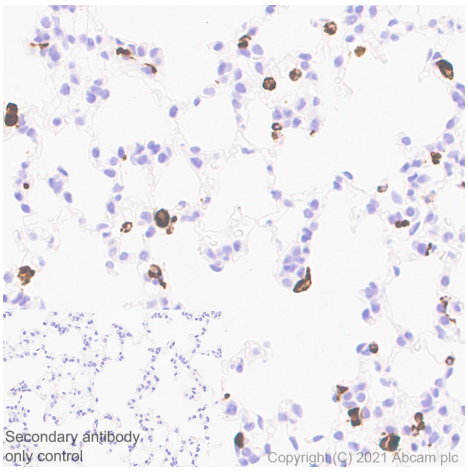
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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



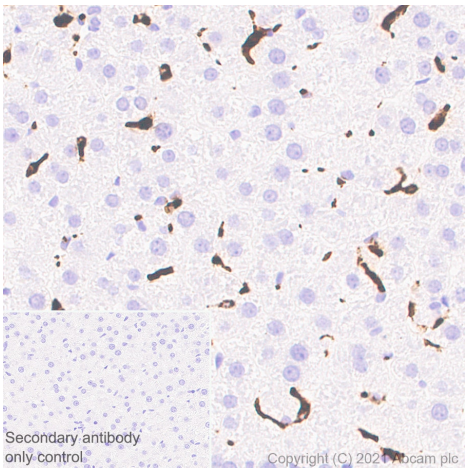
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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



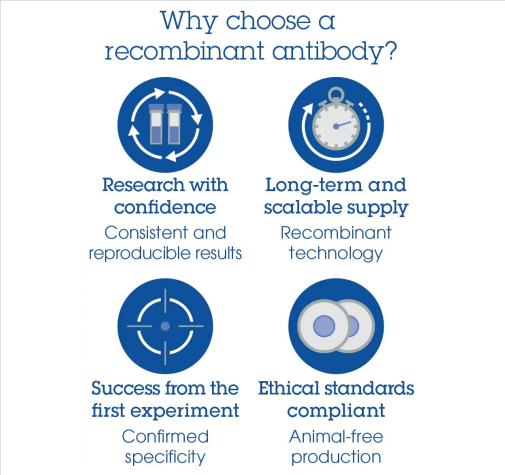
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

This data was developed using **ab283654**, the same antibody clone in a different buffer formulation.

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



Anti-CD68 antibody [EPR23917-164] - BSA and Azide free (ab283667)

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