Product name: Anti-CD68 antibody

Description: Rabbit polyclonal to CD68

Host species: Rabbit

Tested applications: Suitable for: WB, IHC-P, IHC-Fr

Species reactivity: Reacts with: Mouse, Rat

Immunogen: Synthetic peptide corresponding to Mouse CD68 aa 312-326 (internal sequence). Different from the related rat sequence by one amino acid.

Sequence: AFCITRRQSTYQPL

Database link: P31996

Positive control: IHC-P: Rat and mouse liver tissue, Mouse spleen, skin, and brain tissues; IHC-Fr: Rat liver tissue; WB: Wild-type RAW 264.7, Neuro-2a cell lysates, Rat and Mouse spleen tissue lysates. Raw264.7 cell lysate.

General notes: For WB, as CD68 is highly glycosylated, it typically runs between 75-110 kDa depending on the amount of glycosylation in the sample.

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 repeated freeze / thaw cycles.

Storage buffer: Constituents: 0.45% Sodium chloride, 0.1% Dibasic monohydrogen sodium phosphate, 5.61% Trehalose
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

The Abpromise guarantee: Our Abpromise guarantee covers the use of ab125212 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★☆☆☆ (9)</td>
<td>Use a concentration of 0.1 - 0.5 µg/ml. Predicted molecular weight: 35 kDa. The detection limit for ab125212 is approximately 0.1 ng/lane under non-reducing and reducing conditions.</td>
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<tr>
<td>IHC-P</td>
<td>★★★★★☆☆☆ (33)</td>
<td>Use a concentration of 0.5 - 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★☆☆☆ (7)</td>
<td>Use a concentration of 0.5 - 1 µg/ml.</td>
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</table>

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.

Images
All lanes: Anti-CD68 antibody (ab125212) at 0.2 µg/ml

Lane 1: Wild-type RAW 264.7 cell lysate
Lane 2: CD68 knockout RAW 264.7 cell lysate
Lane 3: Mouse spleen cell lysate
Lane 4: Neuro-2a cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 35 kDa
Observed band size: 95-102 kDa

False colour image of Western blot: Anti-CD68 antibody staining at 0.2 µg/ml, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab125212 was shown to bind specifically to CD68. A band was observed at 95-102 kDa in wild-type RAW 264.7 cell lysates with no signal observed at this size in CD68 knockout cell line ab280047 (knockout cell lysate ab280106). To generate this image, wild-type and CD68 knockout RAW 264.7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
Paraffin-embedded rat liver tissue stained for CD68 using ab125212 at 1 μg/ml in immunohistochemical analysis.

ab125212 staining CD68 in Mouse spleen tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 5% serum for 30 minutes at 20°C; antigen retrieval was by heat-mediated in a citrate buffer. Samples were incubated with primary antibody (1/100 in PBS + 2% BSA + 10% FCS) for 45 minutes at 20°C. A HRP-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.
CD68 was detected in paraffin-embedded section of mouse liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1μg/mL ab125212 overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

All lanes: Anti-CD68 antibody (ab125212)

Lane 1: Rat spleen tissue lysate
Lane 2: Mouse spleen tissue lysate
Lane 3: RAW264.7 cell lysate

Predicted band size: 35 kDa
Additional bands at: 90-100 kDa (possible glycosylated form)

Western blot analysis of rat and mouse tissue lysates, labelling CD68 with ab125212
Paraffin-embedded mouse spleen tissue stained for CD68 using ab125212 at 1 μg/ml in immunohistochemical analysis.

Immunohistochemistry analysis of paraffin-embedded rat liver tissue labeling CD68 with ab125212. Heat mediated antigen retrieval was performed in citrate buffer (pH 6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with rabbit anti-CD68 Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1/100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.
**Immunohistochemistry of microglial marker in mouse brain**

Cortical staining for the microglial marker, CD68 increased as a function of age in rTg4510 animals, but remained unchanged in tTA animals. Scale bar, 200 µm, inset scale bar, 50 µm.

(After Figure 10 A of Wes et al.)

Frozen sectioned rat liver tissue stained for CD68 using ab125212 at 1 µg/ml in immunohistochemical analysis.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody (ab125212)
This image is courtesy of an anonymous Abreview.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody (ab125212)
This image is courtesy of an anonymous Abreview.

ab125212 staining CD68 in Mouse skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% serum for 30 minutes at 24°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/2000 in 10% goat serum) for 16 hours at 4°C. A Biotin-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

Immunohistochemical analysis of murine spleen tissue, staining CD68 with ab125212.

Tissue was fixed with paraformaldehyde and blocked with 5% serum for 1 hour at room temperature; antigen retrieval was by heat mediation in citrate buffer (pH 6). Samples were incubated with primary antibody (undiluted) for 16 hours at 4°C. An undiluted HRP-conjugated horse anti-rat polyclonal IgG was used as the secondary antibody.

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