Product datasheet

Anti-CD68 antibody [FA-11] ab53444

Overview

Product name Anti-CD68 antibody [FA-11]
Description Rat monoclonal [FA-11] to CD68
Host species Rat
Specificity ab53444 detects surface CD68 at low levels in resident mouse peritoneal macrophages which can be enhanced with thioglycollate stimulation.
Tested applications Suitable for: ICC/IF, WB, IP, IHC-Fr, Flow Cyt
Species reactivity Reacts with: Mouse
Immunogen Tissue, cells or virus corresponding to Mouse CD68.
Positive control IHC-Fr: Mouse lung, spleen and heart tissue sections; ICC/IF: RAW 246.7 cell line. Flow Cyt: Mouse peritoneal macrophages.
General notes Although some customers have had success with this antibody in IHC-P, we are unable to obtain positive results in this application and so cannot recommend it for IHC-P. We batch test the antibody in IHC-Fr.

This antibody clone is manufactured by Abcam.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form Liquid
Storage buffer pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine
Purity Immunogen affinity purified
Purification notes Purified IgG prepared by affinity chromatography on Protein G from tissue culture supernatant.
Clonality Monoclonal
Clone number FA-11
Isotype IgG2a
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

Applications

Our Abpromise guarantee covers the use of ab53444 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>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆</td>
<td>Use at an assay dependent concentration. Predicted molecular weight: 36 kDa.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★☆</td>
<td>Use a concentration of 0.1 - 5 µg/ml.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/50 - 1/100. Use 10µl of the suggested working dilution to label 10^6 cells in 100µl. Membrane permeabilisation is required for this application.</td>
<td>ab18450 - Rat monoclonal IgG2a, is suitable for use as an isotype control with this antibody.</td>
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Target

Images
Dual fluorescence combining IL-6 with markers for macrophages (CD68).

O, P, and Q, dual labeling of IL-6 (red) and marker of macrophage (green) in db/db mouse heart tissues. Arrows in Q show the specific CD68 staining with absence of IL-6 staining. (R and S) Negative control; arrows show the absence of staining in vessels with control IgG and without primary antibodies. (T) Staining of nuclei with DAPI (blue) in heart tissues of the db/db mice.

To identify and localize IL-6 protein in coronary arterioles, transverse sections of the mouse heart were stained using markers of endothelial cells, vascular smooth muscle cells, and macrophages. Freshly isolated hearts were embedded and frozen in OCT and sectioned at 5 μm. Slides were incubated with blocking solution (10% donkey serum in PBS) and permeabilized (0.1% Triton X-100 in PBS). Primary antibodies to IL-6 (goat polyclonal 15 micro g/ml, AF-406-NA; R&D) or macrophage marker CD68 (rat monoclonal, 1:1000, ab53444; Abcam) were used for sequential double immunofluorescence staining. Secondary antibodies were conjugated with the fluorophores FITC or Texas red. Sections were mounted in an anti-fading agent (Slowfade gold with DAPI; Invitrogen), and then the slides were observed and analyzed with a fluorescence microscope (IX81; Olympus) with a x40 objective (0.90 numerical aperture). For negative controls, primary antibodies were replaced with goat polyclonal IgG (Abcam), rabbit polyclonal IgG (GeneTex), and rat monoclonal IgG (Abcam) isotype controls at the same concentration. The specificity of the primary antibody was confirmed as the absence of immunofluorescence staining signals in the IL-6+/− mice.

Diabetic mice (db/db).

Formaldehyde-fixed, frozen mouse lung tissue sections stained for CD68 using ab53444 at a 1/250 dilution in immunohistochemical analysis. Tissue sections were blocked using 1% BSA as a blocking agent for 10 minutes at 21°C. Primary antibody was incubated for 2 hours at 21°C. Secondary antibody was a biotin-conjugated goat anti-rat IgG at 1/250 dilution.
Immunohistochemistry (Frozen sections) - Anti-CD68 antibody [FA-11] (ab53444)

IHC image of CD68 staining in mouse lung frozen tissue section. The section was incubated with ab53444, 0.1µg/ml, overnight at 4°C. A goat anti-Rat biotinylated secondary antibody was used to detect the primary, and visualized using an ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

ab53444 staining CD68 in mouse spleen tissue by Immunohistochemistry (Frozen sections). Antibody was detected with HRP-conjugated Goat anti-Rat IgG, showing staining in the red pulp.

Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [FA-11] (ab53444)

ab53444 stained in RAW 246.7 cells. 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 for 1 h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab53444 at 1µg/ml and ab7291 (Mouse monoclonal to alpha Tubulin - Loading Control) used at a 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150165, Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed (pseudo-colored green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), preadsorbed (colored red), both used at 1/1000 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.
Staining of permeabilised mouse peritoneal macrophages with Rat anti mouse CD68 (ab53444).

ab53444 staining mouse heart tissue by Immunohistochemistry (IHC-frozen sections). Tissue underwent fixation in paraformaldehyde, no permeabilization and blocked in 1% serum for 10 minutes at 20°C. The primary antibody used undiluted and incubated with sample for 16 hours at 20°C. A Biotin conjugated goat polyclonal to rat Ig, diluted 1/100 was used as the secondary.

ab53444 at 1/100 dilution staining CD68 in mouse spleen tissue by immunohistochemistry (frozen sections). Sections were acetone fixed prior to blocking in 8% milk for 40 minutes at 36°C and then incubated with ab53444 for 20 hours at 4°C. A biotin conjugated goat polyclonal to rat Ig, diluted 1/400, was used as the secondary antibody.
ab53444 staining CD68 in murine heart tissue by Immunohistochemistry (Frozen sections).

Tissue was fixed in acetone, permeabilized using 0.3% Triton, blocked with 10% serum for 30 minutes at 20°C, then incubated with ab53444 at a 1/1000 dilution for 16 hours at 4°C. The secondary used was a FITC conjugated goat anti-rat polyclonal, used at a 1/1000 dilution.

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