Overview

Product name: Anti-CD68 antibody [KP1]
Description: Mouse monoclonal [KP1] to CD68
Host species: Mouse
Tested applications: Suitable for: ICC/IF, WB, IHC-P
Species reactivity: Reacts with: Human

Immunogen: Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers.
Positive control: IHC-P: human tonsil and liver tissues; ICC/IF: THP-1 cells; WB: Human spleen lysate, THP-1 whole cell lysate

General notes:
- Anti CD68 antibody (ab955) is recommended for IHC on human samples but is not recommended for mouse & rat samples.
- This product has switched from a hybridoma to recombinant production method on 21st September 2020. The concentration listed relates to the purified version, please contact Scientific Support for unpurified concentration.
- 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.

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer: Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity: Protein A purified
Clonality: Monoclonal
Clone number: KP1
Myeloma: unknown
Isotype: IgG1
Light chain type: kappa

Applications

The Abpromise guarantee covers the use of ab955 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>⭐⭐⭐⭐⭐ (5)</td>
<td>1/50.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ (1)</td>
<td>1/1000. Predicted molecular weight: 37 kDa.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐ (29)</td>
<td>1/3000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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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.


Images
Western blot - Anti-CD68 antibody [KP1] (ab955)

Lanes 1 & 3 : Anti-CD68 antibody [KP1] (ab955) at 1/1000 dilution
Lanes 2 & 4 : Anti-CD68 antibody [RM1031] (ab303565) at 1/1000 dilution

Lanes 1-2 : Human spleen tissue lysate
Lanes 3-4 : THP-1 (Human monocytic leukemia monocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 37 kDa
Observed band size: 110 kDa

Blocking and diluting buffer and concentration: 5% NFDM /TBST
Exposure time: Lane1: 60 seconds; Lane2: 5 seconds; Lane3: 180 seconds; Lane4: 7 seconds

ab303565 works better than ab955 in western blot testing.

We suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate) to improve results when using ab955 in western blot.
Immunocytochemistry analysis of THP-1 (human monocytic leukemia monocyte) labelling CD68 with ab955 at 1/50 dilution. Cells were fixed with 100% methanol. Goat Anti-mouse IgG H&L (Alexa Fluor® 488) (ab150113) at 1/1000 was used as the secondary antibody (green). Cells were counterstained with Anti-Tubulin antibody (rabbit mAb), ab179504 - AlexaFluor®594 Goat anti-Rabbit secondary, ab150080 at 1/500 dilution (red). Nuclear DNA was labelled with DAPI (blue). 

Confocal image showing cytoplasmic staining in THP-1 cells

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human liver tissue labelling CD68 with ab955 at 1/3000 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab93684 a Goat Anti-mouse IgG H&L (HRP) was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin. Cytoplasmic staining on Kupffer cells of human liver (PMID: 12118106).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue labelling CD68 with ab955 at 1/3000 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab93684** a Goat Anti-mouse IgG H&L (HRP) was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Cytoplasmic staining on macrophages of human tonsil (PMID: 19543531).

**ab955 staining CD68 in human liver tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).**

Tissue was fixed with paraformaldehyde. Samples were then blocked with 10% serum for 3 hours at 22°C followed by incubation with the primary antibody at a 1/100 dilution for 16 hours at 4°C. An Alexa-Fluor® 568 conjugated goat anti-mouse polyclonal was used as secondary antibody at a 1/400 dilution.
ab955 staining CD68 in human ulcerated oral (Mucosa/Bone) tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with paraformaldehyde, permeabilized with 0.1% Triton-X 100 in PBS and blocked with 2.5% serum for 90 minutes at 25°C; antigen retrieval was by heat mediation in citrate buffer (pH 6). Samples were incubated with primary antibody (1/500 in 1% serum in PBS +0.01% Triton-X 100) for 16 hours at 4°C. A commercial IHC kit and DAB was used to visualize the staining.

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