# abcam

## Product datasheet

## Anti-CD68 antibody [KP1] - BSA and Azide free ab233172





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#### Overview

**Product name** Anti-CD68 antibody [KP1] - BSA and Azide free

**Description** Mouse monoclonal [KP1] to CD68 - BSA and Azide free

**Host species** Mouse

**Tested applications** Suitable for: Mass Cytometry, ICC/IF, IHC-P, WB

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Rabbit

Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control WB: Human spleen lysate, THP-1 whole cell lysate IHC-P: Human tonsil and liver tissue. ICC/IF:

THP-1 cells. IMC: Human tonsil tissue

**General notes** ab233172 is the carrier-free version of ab955.

> 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 cellbased 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<sup>®</sup> is a trademark of Fluidigm Canada Inc.

#### **Properties**

**Form** Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

**Purity** Protein A purified

**Clonality** Monoclonal

Clone number KP1
Isotype IgG1
Light chain type kappa

## **Applications**

The Abpromise guarantee

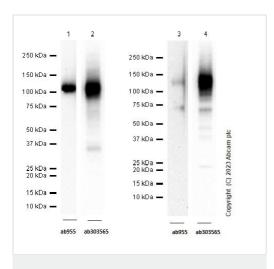
Our  $\underline{\textbf{Abpromise guarantee}}$  covers the use of ab233172 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Mass Cytometry	<b>★★★★ (1)</b>	Use at an assay dependent concentration.
ICC/IF		1/50.
IHC-P		1/3000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 37 kDa).

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 [KP1] - BSA and Azide free (ab233172)

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

 $\begin{tabular}{ll} \textbf{All lanes:} Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution \end{tabular}$ 

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

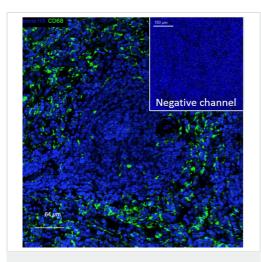
This data was developed using the same antibody clone in a different buffer formulation (ab955).

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

Exposure time: Lane1: 60 seconds; Lane2: 5 seconds; Lane3: 180 seconds; Lane4: 7 seconds

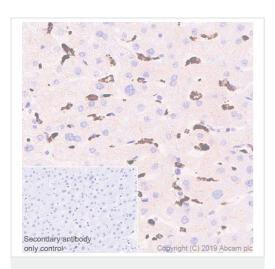
ab303565 works better than ab955 in western blot testing.

We suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate) to improve results when using <u>ab955</u> in western blot.



Mass Cytometry - Anti-CD68 antibody [KP1] - BSA and Azide free (ab233172)

This image is courtesy of the Single Cell & Imaging Mass Cytometry Analysis Platform, Goodman Cancer Research Centre, McGill University



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [KP1] - BSA and Azide free (ab233172)

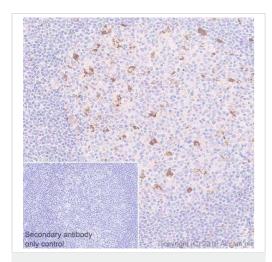
Imaging Mass Cytometry™ (IMC™) image of human tonsil tissue stained with Anti-CD68 antibody [KP1]. ab233172 (carrier-free antibody, purified) was metal-conjugated using a Maxpar® Antibody Labeling Kit from Fluidigm. Immunostaining was performed according to Fluidigm's protocols. Briefly, slides were subject to deparaffinization and heat-induced epitope retrieval, followed by overnight incubation at 4°C with an antibody cocktail containing metal-tagged antibodies in blocking buffer. Slides were subsequently washed with 0.2% Triton-X and 1x PBS, counterstained with Cell-ID™ Intercalator-Ir diluted at 1/400 in 1x PBS for 30 min at room temperature, rinsed for 5 min with distilled H2O, and air-dried prior to IMC ™ acquisition. IMC ™ acquisition was performed using the Fluidigm Hyperion™ Imaging System.

Imaging Mass Cytometry™, IMC™, Cell-ID™, Hyperion™ and Maxpar® are trademarks of Fluidigm Canada

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CD68 with ab233172 at 1/3000 dilution, followed by Goat Anti-mouse IgG H&L (HRP) ready to use. Cytoplasmic staining on Kupffer cells of human liver (PMID: 12118106) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-mouse IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

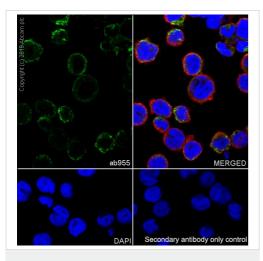


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD68 antibody [KP1] - BSA and Azide free (ab233172)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD68 with ab233172 at 1/3000 dilution, followed by Goat Anti-mouse IgG H&L (HRP) ready to use. Cytoplasmic staining on macrophages of human tonsil (PMID: 19543531) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-mouse IgG H&L (HRP) ready to use.

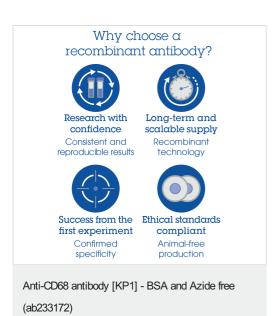
Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [KP1] - BSA and Azide free (ab233172)

Immunofluorescent analysis of 100% methanol-fixed THP-1 (human monocytic leukemia cell line) cells labeling CD68 with ab233172 at 1/50 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (ab150113) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in THP-1 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with Recombinant Anti-beta IV Tubulin antibody [EPR16775] (ab179504) at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (ab150080) secondary antibody at 1/1000 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (ab150113) secondary antibody at 1/1000 dilution.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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