Anti-CD68 Antibody [PSH05-47] - BSA and Azide free

HA751006

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	PSH05-47

Description: CD68 (Cluster of Differentiation 68) is a protein highly expressed by cells in the monocyte lineage (e.g., monocytic phagocytes, osteoclasts), by circulating macrophages, and by tissue macrophages (e.g., Kupffer cells, microglia). Human CD68 is a Type I transmembrane glycoprotein, heavily glycosylated in its extracellular domain, with a molecular weight of 110 kD. Its primary sequence consists of 354 amino acids with predicted molecular weight of 37.4 kD if it were not glycosylated. The human CD68 protein is encoded by the "CD68" gene which maps to Chromosome 17. Other names or aliases for this gene in humans and other animals include: CD68 Molecule, CD68 Antigen, GP110, Macrosialin, Scavenger Receptor Class D, Member 1, SCARD1, and LAMP4. The mouse equivalent is known as "macrosialin".

Immunogen: Recombinant protein within Mouse CD68 aa 21-326.

Positive control: Mouse spleen tissue, rat spleen tissue, RAW264.7 cell lysate, M NFS 60 cell lysate, J774A.1 cell lysate, Rat spleen tissue lysate, RAW264.7, BV2.

Subcellular location: Cell membrane; Endosome membrane, Lysosome membrane.

Database links: SwissProt: P31996 Mouse
Entrez Gene: 287435 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:100-1:200
IHC-P	1:2,000
IHC-Fr	1:500
IF-Tissue	1:200

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

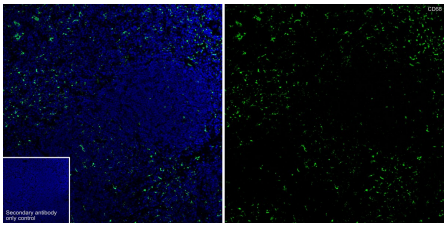

Fig1: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-CD68 antibody (HA751006) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA751006, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

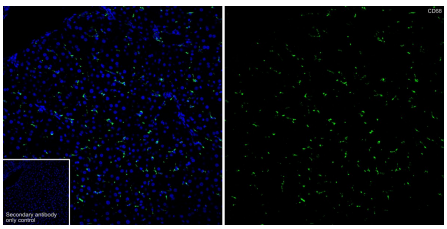

Fig2: Immunofluorescence analysis of frozen mouse liver tissue with Rabbit anti-CD68 antibody (HA751006) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA751006, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

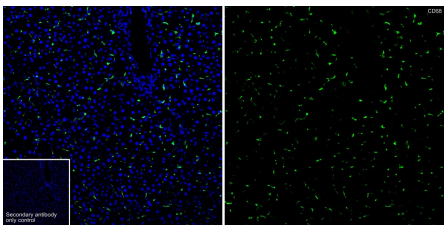

Fig3: Immunofluorescence analysis of frozen rat liver tissue with Rabbit anti-CD68 antibody (HA751006) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA751006, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

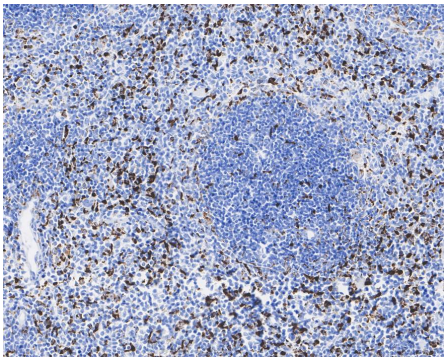

Fig4: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD68 antibody (HA751006) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751006) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

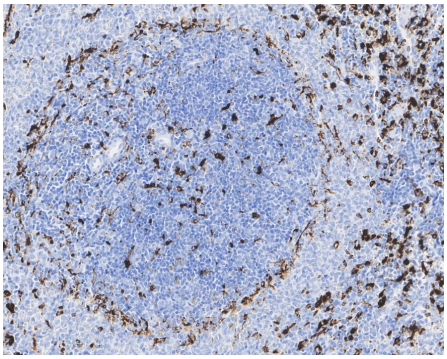

Fig5: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-CD68 antibody (HA751006) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751006) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

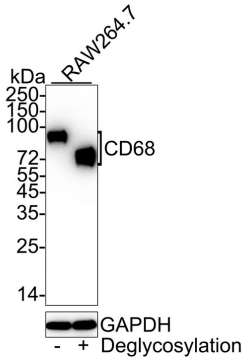

Fig6: Western blot analysis of CD68 on different lysates with Rabbit anti-CD68 antibody (HA751006) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 cell lysate treated with deglycosylation

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 90/70 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751006) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

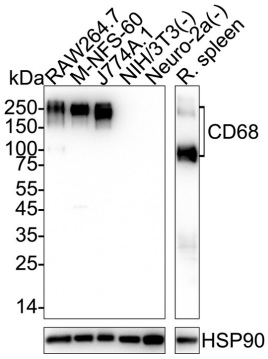

Fig7: Western blot analysis of CD68 on different lysates with Rabbit anti-CD68 antibody (HA751006) at 1/5,000 dilution.

Lane 1: RAW264.7 cell lysate (20 µg/Lane)
Lane 2: M NFS 60 cell lysate (20 µg/Lane)
Lane 3: J774A.1 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (negative) (20 µg/Lane)
Lane 5: Neuro-2a cell lysate (negative) (20 µg/Lane)
Lane 6: Rat spleen tissue lysate (no heat) (40 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 35 kDa
Observed band size: 90-200 kDa

Exposure time: Lane 1-5: 12 seconds; Lane 6: 40 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751006) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

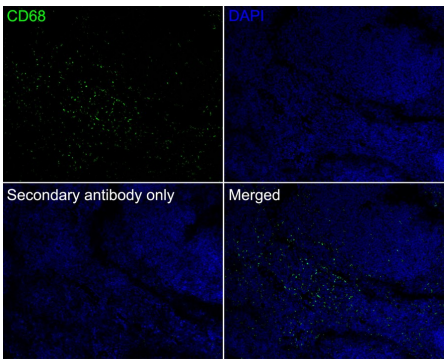

Fig8: Immunofluorescence analysis of paraffin-embedded mouse spleen tissue labeling CD68 with Rabbit anti-CD68 antibody (HA751006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA751006, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

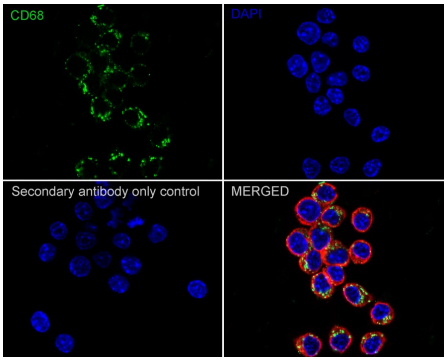

Fig9: Immunocytochemistry analysis of RAW264.7 cells labeling CD68 with Rabbit anti-CD68 antibody (HA751006) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD68 antibody (HA751006) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

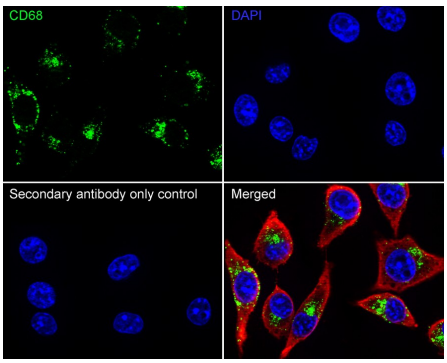

Fig10: Immunocytochemistry analysis of BV2 cells labeling CD68 with Rabbit anti-CD68 antibody (HA751006) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD68 antibody (HA751006) at 1/200 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Deng R et al. Periosteal CD68(+) F4/80(+) Macrophages Are Mechanosensitive for Cortical Bone Formation by Secretion and Activation of TGF-beta1. Adv Sci (Weinh). 2022 Jan
2. Duan L et al. Myeloid-restricted CD68 deficiency attenuates atherosclerosis via inhibition of ROS-MAPK-apoptosis axis. Biochim Biophys Acta Mol Basis Dis. 2023 Jun