Anti-CD68 Antibody [A3C3-R]

HA601290

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 37 kDa
Clone number:	A3C3-R

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 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. Immunohistochemistry can be used to identify the presence of CD68, which is found in the cytoplasmic granules of a range of different blood cells and myocytes. It is particularly useful as a marker for the various cells of the macrophage lineage, including monocytes, histiocytes, giant cells, Kupffer cells, and osteoclasts. This allows it to be used to distinguish diseases of otherwise similar appearance, such as the monocyte/macrophage and lymphoid forms of leukaemia (the latter being CD68 negative). Its presence in macrophages also makes it useful in diagnosing conditions related to proliferation or abnormality of these cells, such as malignant histiocytosis, histiocytic lymphoma, and Gaucher's disease. Anti-CD68 monoclonal antibodies that react with tissues of rodent and other species include ED1, FA-11, KP1 (a.k.a. C68/684), 6A326, 6F3, 12E2, 10B1909, and SPM130. Monoclonals that react with humans include, Ki-M7, PG-M1, 514H12, ABM53F5, 3F7C6, 3F7D3, Y1/82A, EPR20545, CDLA68-1, LAMP4-824.

Immunogen: Synthetic peptide within human CD68 aa 320-354.

Positive control: THP-1 cell lysate, U-937 cell lysate, U-87 MG cell lysate, THP-1, human lung cancer tissue, human prostate cancer tissue, human tonsil tissue.

Subcellular location: Cell membrane. Endosome membrane, lysosome membrane.

Database links: SwissProt: P34810 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IHC-P	1:1,000
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

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

Purity: Protein A affinity purified.

Images

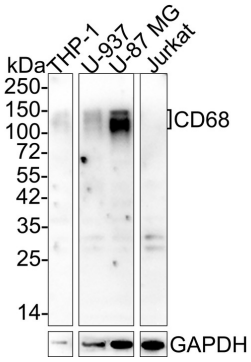

Fig1: Western blot analysis of CD68 on different lysates with Mouse anti-CD68 antibody (HA601290) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: U-937 cell lysate
Lane 3: U-87 MG cell lysate
Lane 4: Jurkat cell lysate (negative)

Lysates/proteins at 10 µg/Lane.

Predicted band size: 37 kDa
Observed band size: 100-150 kDa

Exposure time: 2 minutes 45 seconds;

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 (HA601290) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

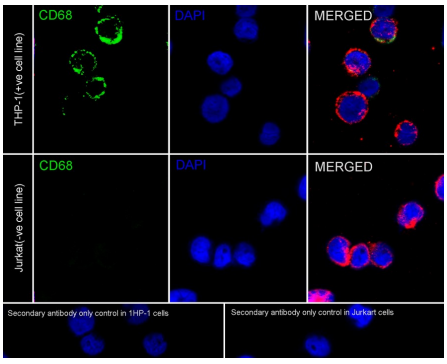

Fig2: Immunocytochemistry analysis of THP-1 (positive) and Jurkat (negative) labeling CD68 with Mouse anti-CD68 antibody (HA601290) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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 Mouse anti-CD68 antibody (HA601290) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

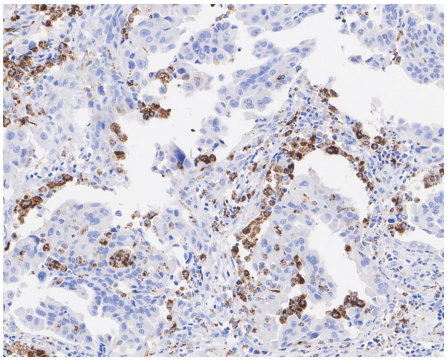

Fig3: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Mouse anti-CD68 antibody (HA601290) at 1/1,000 dilution.

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 (HA601290) at 1/1,000 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.

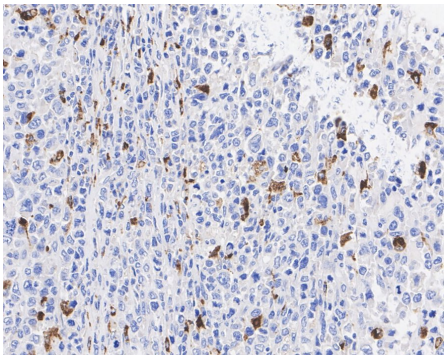

Fig4: Immunohistochemical analysis of paraffin-embedded human prostate cancer tissue with Mouse anti-CD68 antibody (HA601290) at 1/1,000 dilution.

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 (HA601290) at 1/1,000 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.

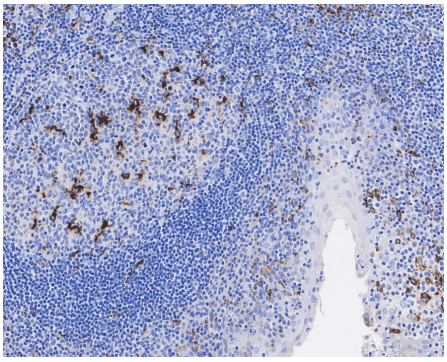

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Mouse anti-CD68 antibody (HA601290) at 1/1,000 dilution.

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 (HA601290) at 1/1,000 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.

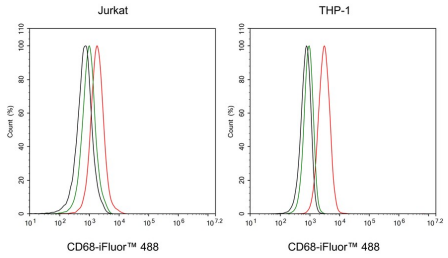

Fig6: Flow cytometric analysis of THP-1 (positive) and Jurkat (negative) cells labeling CD68.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601290, 1μg/mL) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Wang L. et. al. Specific clinical and immune features of CD68 in glioma via 1,024 samples. Cancer Manag Res. 2018 Nov 27;10:6409-6419.
2. Minami K. et. al. Prognostic significance of CD68, CD163 and Folate receptor-β positive macrophages in hepatocellular carcinoma. Exp Ther Med. 2018 May;15(5):4465-4476.