Anti-CD68 Antibody [A3C4]

EM1901-95

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, FC, mIHC
Molecular Wt:	Predicted band size: 37 kDa.
Clone number:	A3C4

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:	A431 cell lysate, THP-1 cell lysate, U-937 cell lysate, human tonsil tissue, human lung tissue, THP-1, human pancreatic carcinoma.
Subcellular location:	Cell membrane. Endosome membrane, lysosome membrane.
Database links:	SwissProt: P34810 Human
Recommended Dilutions:	
WB	1:1,000-1:2,000
IHC-P	1:50-1:200
FC	1:50-1:100
mIHC	1:3,000
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein G affinity purified.

Images

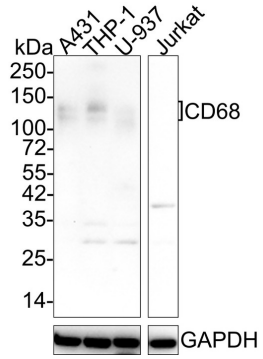

Fig1: Western blot analysis of CD68 on different lysates with Mouse anti-CD68 antibody (EM1901-95) at 1/1,000 dilution.

Lane 1: A431 cell lysate
Lane 2: THP-1 cell lysate
Lane 3: U-937 cell lysate
Lane 4: Jurkat cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 3 minutes;

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 (EM1901-95) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

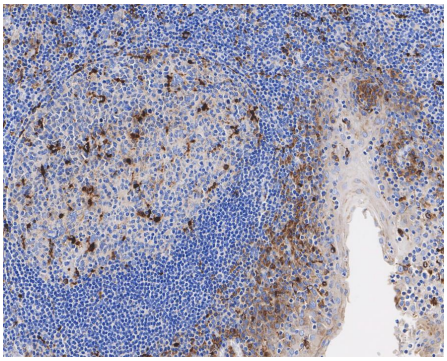

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD68 antibody (EM1901-95) at 1/1,000 dilution.

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 (EM1901-95) 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.

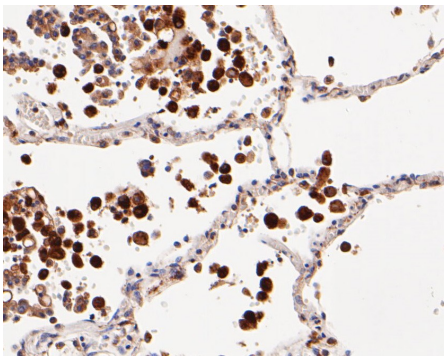

Fig3: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-CD68 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-95, 1/200) for 30 minutes 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.

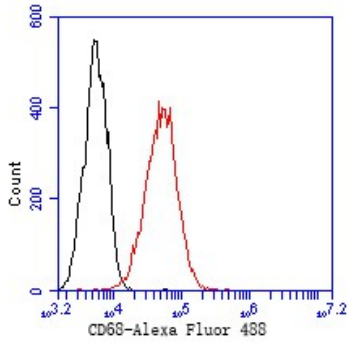

Fig4: Flow cytometric analysis of CD68 was done on THP-1 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-95, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

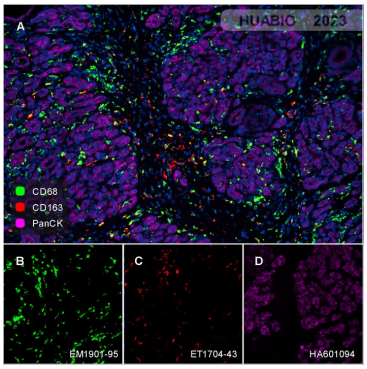

Fig5: Fluorescence multiplex immunohistochemical analysis of the human pancreatic carcinoma (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (EM1901-95, green), anti-CD163 (ET1704-43, red) and anti-PanCK (HA601094, violet) on human pancreatic carcinoma. Panel B: anti- CD68 stained on M1 macrophages. Panel C: anti-CD163 stained on M2 macrophages cells. Panel D: anti-panCK stained on cancer cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of EM1901-95 (1/3,000 dilution), ET1704-43 (1/3,000 dilution), and HA601094 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Nikon ECLIPSE Ni-E microscope.

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.