Anti-CD68 Antibody [PDM0-13]

HA601115

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	IHC-P, IF-Cell, FC, mIHC, IF-Tissue
Molecular Wt:	Predicted band size: 37 kDa
Clone number:	PDM0-13

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.

Immunogen: Recombinant protein within Human CD68 aa 1-354.

Positive control: Human spleen tissue, human diffuse large B-cell lymphoma tissue, human tonsil tissue, human liver tissue, THP-1, human non-small cell lung cancer, human cervical cancer.

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

Database links: SwissProt: P34810 Human

Recommended Dilutions:

IHC-P	1:2,000-1:4,000
IF-Cell	1:100
FC	1:500-1:1,000
mIHC	1:1,500-1:2,000
IF-Tissue	1:50-1:800

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

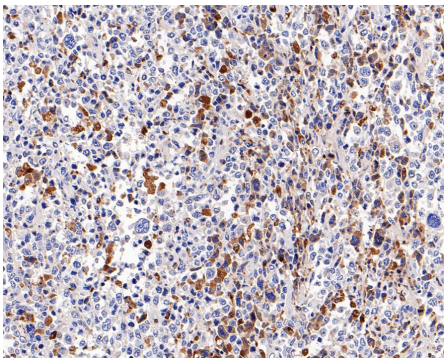

Fig1: Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue with Mouse anti-CD68 antibody (HA601115) at 1/4,000 dilution.

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 (HA601115) at 1/4,000 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.

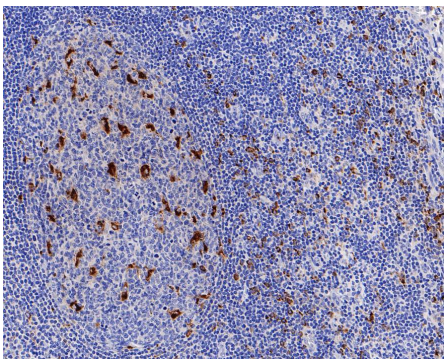

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Mouse anti-CD68 antibody (HA601115) at 1/2,000 dilution.

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 (HA601115) 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.

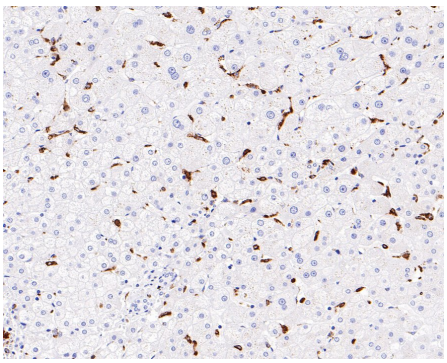

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-CD68 antibody (HA601115) at 1/4,000 dilution.

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 (HA601115) at 1/4,000 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.

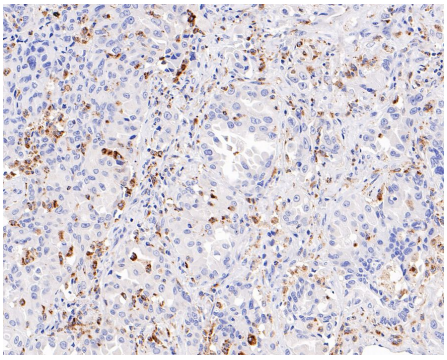

Fig4: Immunohistochemical analysis of paraffin-embedded human lung adenocarcinoma tissue (Negative control) with Mouse anti-CD68 antibody (HA601115) at 1/4,000 dilution.

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 (HA601115) at 1/4,000 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.

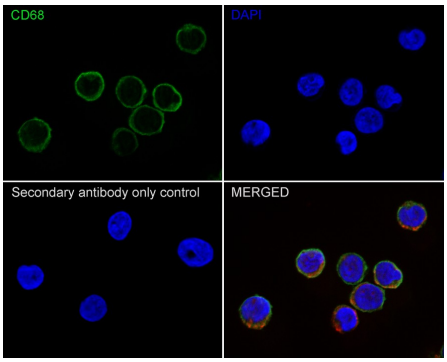

Fig5: Immunocytochemistry analysis of THP-1 cells labeling CD68 with Mouse anti-CD68 antibody (HA601115) at 1/100 dilution.

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 Mouse anti-CD68 antibody (HA601115) 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.

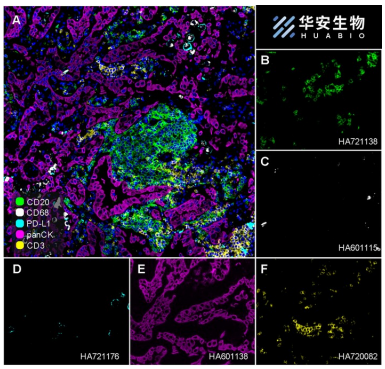

Fig6: Fluorescence multiplex immunohistochemical analysis of the human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD68 (HA601115, gray), anti-PD-L1 (HA721176, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human non-small cell lung cancer. Panel B: anti- CD20 stained on B cells. Panel C: anti-CD68 stained on macrophage M1 and macrophage M2. Panel D: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T 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 five rounds of staining: in the order of HA721138 (1/1,500 dilution), HA601115 (1/2,000 dilution), HA721176 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (1/500 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

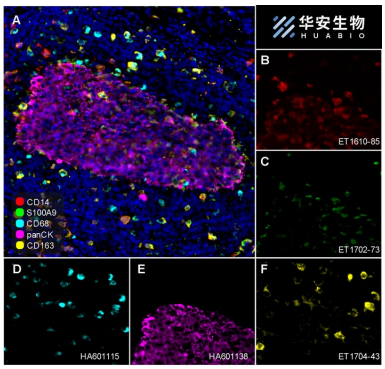

Fig7: Fluorescence multiplex immunohistochemical analysis of the human cervical cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (ET1610-85, red), anti-S100A9 (ET1702-73, green), anti-CD68 (HA601115, cyan), anti-panCK (HA601138, magenta) and anti-CD163 (ET1704-43, yellow) on human cervical cancer. Panel B: anti-CD14 stained on monocyte and MDSCs. Panel C: anti-S100A9 stained on MDSCs. Panel D: anti-CD68 stained on macrophage M1 and macrophage M2. Panel E: anti-panCK stained on tumor cells. Panel F: anti-CD163 stained on macrophage M2. 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 five rounds of staining: in the order of ET1610-85 (1/1,000 dilution), ET1702-73 (1/1,000 dilution), HA601115 (1/2,000 dilution), HA601138 (1/3,000 dilution), and ET1704-43 (1/2,000 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 Olympus VS200 Slide Scanner.

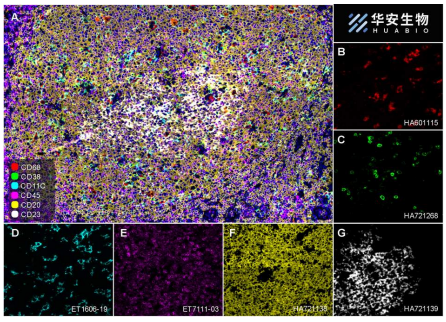

Fig8: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (HA601115, Red), anti-CD38 (HA721268, Green), anti-CD23 (HA721139, White), anti-CD11C (ET1606-19, Cyan), anti-CD45 (ET7111-03, Magenta) and anti-CD20 (HA721138, Yellow) on tonsil. Panel B: anti-CD68 stained on Macrophage. Panel C: anti-CD38 stained on lymphocyte subsets. Panel D: anti-CD11C stained on dendritic cells. Panel E: CD45 stained on lymphocytes. Panel F: anti-CD20 stained on B cells. Panel G: anti-CD23 stained on follicular dendritic 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 six rounds of staining: in the order of HA601115 (1/2,000 dilution), HA721268 (1/1,000 dilution), ET1606-19 (1/1,000 dilution), ET7111-03 (1/500 dilution), HA721138 (1/2,000 dilution) and HA721139 (1/800 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 Olympus VS200 Slide Scanner.

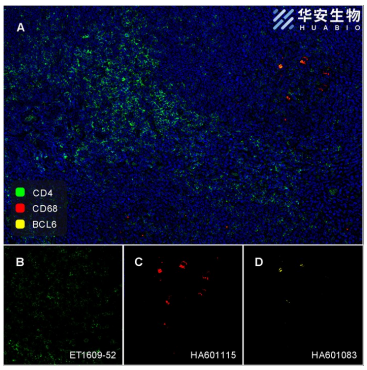

Fig9: Fluorescence multiplex immunohistochemical analysis of human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (HA601115, Red), anti-BCL6 (HA601083, Yellow) and anti-CD4 (ET1609-52, Green) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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 HA601115 (1/2,000 dilution), HA601083 (1/200 dilution) and ET1609-52 (1/800 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 Zeiss Observer 7 Inverted Fluorescence Microscope.

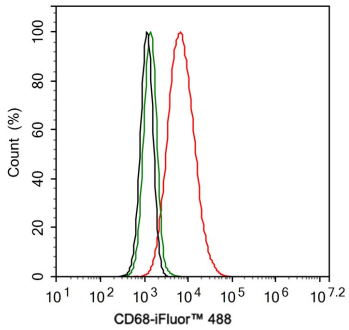

Fig10: Flow cytometric analysis of THP-1 cells labeling CD68.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601115, 1ug/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).

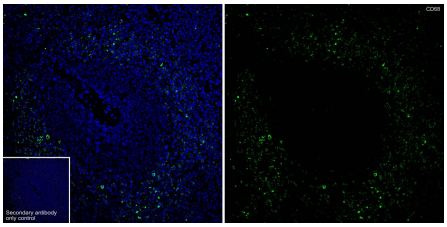

Fig11: Immunofluorescence analysis of paraffin-embedded human spleen tissue labeling CD68 with Mouse anti-CD68 antibody (HA601115) at 1/500 dilution.

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 (HA601115, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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.

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation