Anti-CD163 Antibody [JA51-30]

ET1704-43

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IP, FC, mIHC
Molecular Wt:	Predicted band size: 125 kDa
Clone number:	JA51-30

Description: CD163, also designated M130, is a macrophage-associated antigen that is a member of the scavenger receptor cysteine-rich (SRCR) superfamily. It is highly expressed on macrogphages and to a lesser extent on monocytes. The acute phase-regulated and signal-inducing macrophage protein, CD163, is a receptor that scavenges hemoglobin by mediating endocytosis of haptoglobin-hemoglobin complexes. CD163 binds only haptoglobin and hemoglobin in complex, which indicates the exposure of a receptor-binding neoepitope. The receptor-ligand interaction is calcium-dependent and of high affinity. The existence of several CD163 isoforms, which differ in the structure of their cytoplasmic domains and putative phosphorylation sites, suggests that these isoforms also differ in their signaling mechanism. The gene which encodes CD163 maps to human chromosome 12p13.31.

Immunogen: Recombinant protein within Human CD163 aa 1012-1149 / 1156.

Positive control: Human pancreatic carcinoma, human cervical cancer, human lung tissue lysates, human liver tissue lysates, human liver tissue, human spleen tissue, human placenta tissue, human tonsil tissue, THP-1.

Subcellular location: Secreted, Cell membrane.

Database links: SwissProt: Q86VB7 Human

Recommended Dilutions:

WB	1:500-1:2,000
IHC-P	1:1,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.
mIHC	1:2,000-1:3,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

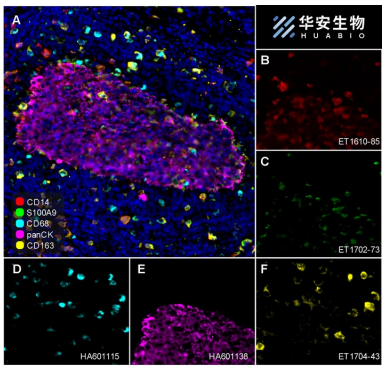

Fig1: Fluorescence multiplex immunohistochemical analysis of the human cervical cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (ET1610-85, red), anti-S100A9 (ET1702-73, green), anti-CD68 (HA601115, cyan), anti-panCK (HA601138, magenta) and anti-CD163 (ET1704-43, 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.

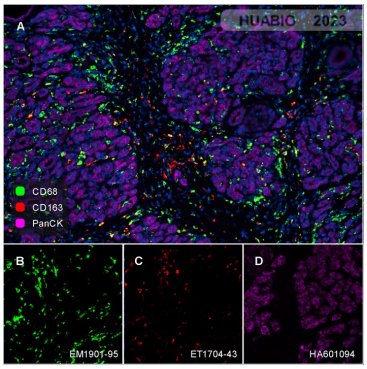

Fig2: Fluorescence multiplex immunohistochemical analysis of the human pancreatic carcinoma (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (EM1901-95, green), anti-CD163 (ET1704-43, red) and anti-PanCK (HA601094, violet) on human pancreatic carcinoma. Panel B: 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.

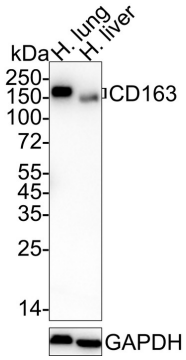

Fig3: Western blot analysis of CD163 on different lysates with Rabbit anti-CD163 antibody (ET1704-43) at 1/1,000 dilution.

Lane 1: Human lung tissue lysate
Lane 2: Human liver tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 125 kDa
Observed band size: 150-170 kDa

Exposure time: 1 minute; 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 (ET1704-43) 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.

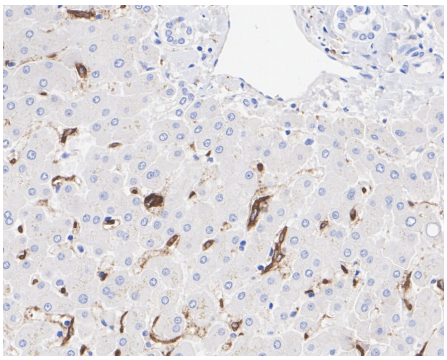

Fig4: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-CD163 antibody (ET1704-43) at 1/1,000 dilution.

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 (ET1704-43) 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.

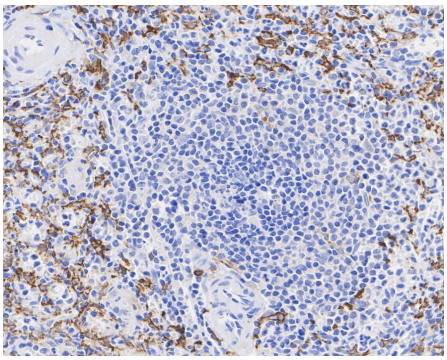

Fig5: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD163 antibody (ET1704-43) at 1/1,000 dilution.

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 (ET1704-43) 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.

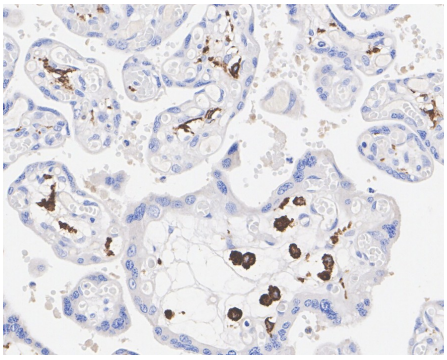

Fig6: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-CD163 antibody (ET1704-43) at 1/1,000 dilution.

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 (ET1704-43) 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.

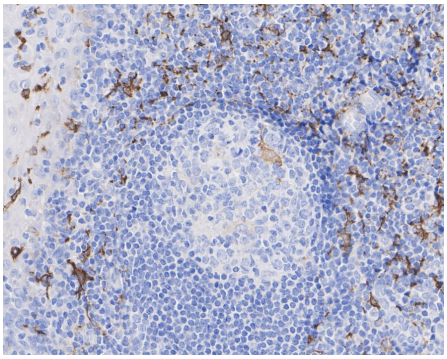

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD163 antibody (ET1704-43) at 1/1,000 dilution.

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 (ET1704-43) 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.

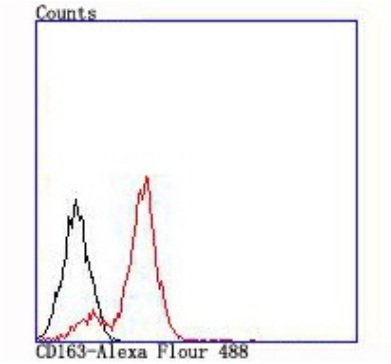

Fig8: Flow cytometric analysis of CD163 was done on THP-1 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1704-43, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

1. Sato Y et al. The PD-1/PD-L1 axis may be aberrantly activated in occupational cholangiocarcinoma.Pathol Int 67(3):163-170 (2017).
2. Chen H et al. An Agonist of the Protective Factor SIRT1 Improves Functional Recovery and Promotes Neuronal Survival by Attenuating Inflammation after Spinal Cord Injury. J Neurosci 37:2916-2930 (2017).