Anti-CD14 Antibody [SC69-02] - BSA and Azide free

HA750232

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 40 kDa
Clone number:	SC69-02

Description: Lipopolysaccharide (LPS) elicits the secretion of mediators and cytokines produced by activated macrophages and monocytes. CD14 is a glycosylphosphatidylinositol (GPI)-anchored protein found on the surfaces of monocytes and polymorphonuclear leukocytes. CD14 functions as a receptor for LPS, resulting in the secretion of various proteins. An important component in the LPS activation of monocytes through the CD14 receptor is the "adapter molecule," lipopolysaccharide binding protein (LBP). There are two forms of CD14, a membrane-associated form (mCD14), and a soluble form (sCD14). mCD14 responds to LPS alone and facilitates the secretion of proteins, while cells not expressing mCD14 fail to respond to LPS. The cells that lack mCD14 respond to LPS/LBP in the presence of sCD14.

Immunogen: Synthetic peptide within Human CD14 aa 310-335 / 375.

Positive control: THP-1 cell lysate, RAW264.7 cell lysate, NIH/3T3 cell lysates, A549, NCCIT, NIH/3T3, LO2, human tonsil tissue, human liver tissue, human colon carcinoma tissue, human spleen tissue, human uterus tissue, human lymph nodes tissue, human cervical cancer.

Subcellular location: Cell membrane, Secreted, Golgi apparatus, Membrane raft.

Database links: SwissProt: P08571 Human | P10810 Mouse

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IHC-P	1:200-1:800
mIHC	1:800-1:1,000
FC	1:1,000

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

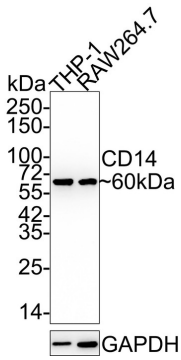

Fig1: Western blot analysis of CD14 on different lysates with Rabbit anti-CD14 antibody (HA750232) at 1/5,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: RAW264.7 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 40 kDa
Observed band size: 60 kDa

Exposure time: 1 minute 40 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 (HA750232) 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.

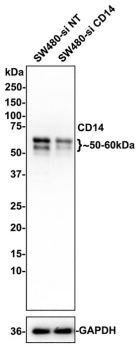

Fig2: Western blot analysis of CD14 on different lysates with Rabbit anti-CD14 antibody (HA750232) at 1/1,000 dilution.

Lane 1: SW480-si NT cell lysate
Lane 2: SW480-si CD14 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 40 kDa
Observed band size: 50-60 kDa

Exposure time: 7 seconds; ECL: merk
4-20% SDS-PAGE gel.

ET1610-85 was shown to specifically react with CD14 in SW480-si NT cells. Weakened band was observed when SW480-si CD14 sample was tested. SW480-si NT and SW480-si CD14 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1610-85, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

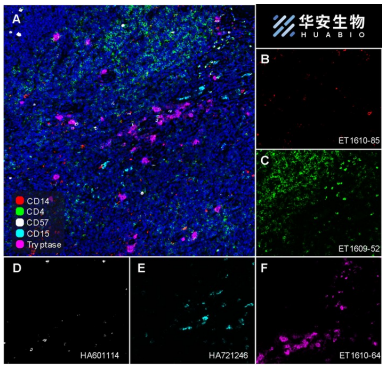

Fig3: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (HA750232, Red), anti-CD4 (ET1609-52, Green), anti-CD57 (HA601114, White), anti-CD15 (HA721246, Cyan)and anti-Tryptase (ET1610-64, Magenta) on tonsil. Panel B: anti- CD14 stained on monocytes. Panel C: anti-CD4 stained on helper T cells and Treg cells. Panel D: anti-CD57 stained on NK cells and T cells. Panel E: CD15 stained on granulocytes and monocytes. Panel F: anti-Tryptase stained on Mast 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 ET1610-85 (1/800 dilution), ET1609-52 (1/800 dilution), HA601114 (1/1,000 dilution), HA721246 (1/500 dilution), and ET1610-64 (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 Olympus VS200 Slide Scanner.

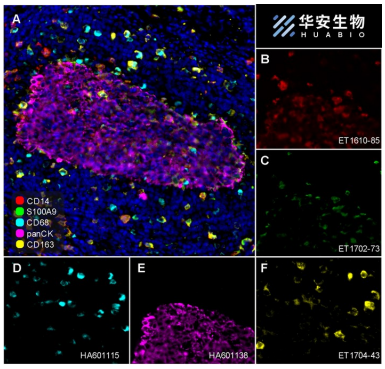

Fig4: Fluorescence multiplex immunohistochemical analysis of the human cervical cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (HA750232, red), anti-S100A9 (ET1702-73, green), anti-CD68 (HA601115, cyan), anti-panCK (HA601138, magenta) and anti-CD163 (ET1704-43, 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.

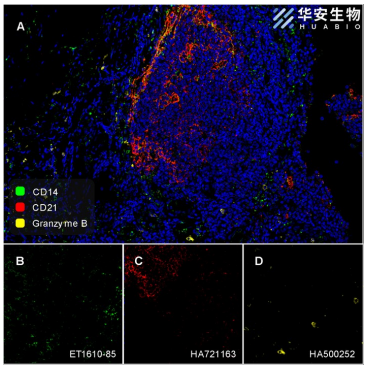

Fig5: Fluorescence multiplex immunohistochemical analysis of human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (HA750232, Green), anti-CD21 (HA721163, Red) and anti-Granzyme B (HA500252, Yellow) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1610-85 (1/800 dilution), HA721163 (1/1,000 dilution) and HA500252 (1/200 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.

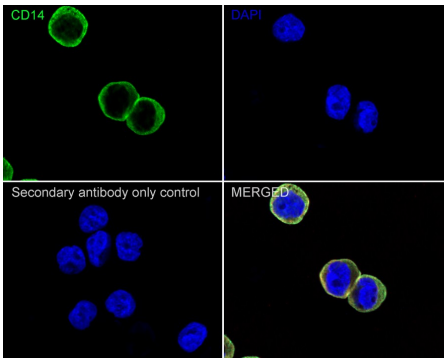

Fig6: Immunocytochemistry analysis of THP-1 cells labeling CD14 with Rabbit anti-CD14 antibody (HA750232) at 1/100 dilution.

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-CD14 antibody (HA750232) 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.

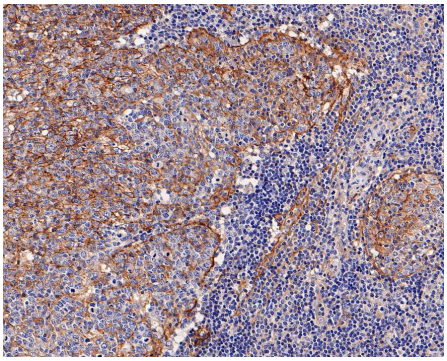

Fig7: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD14 antibody (HA750232) at 1/800 dilution.

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 (HA750232) at 1/800 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.

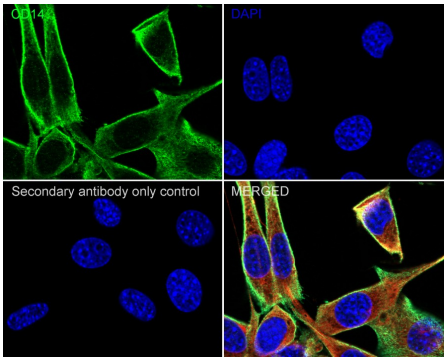

Fig8: Immunocytochemistry analysis of NIH/3T3 cells labeling CD14 with Rabbit anti-CD14 antibody (HA750232) at 1/100 dilution.

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-CD14 antibody (HA750232) 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.

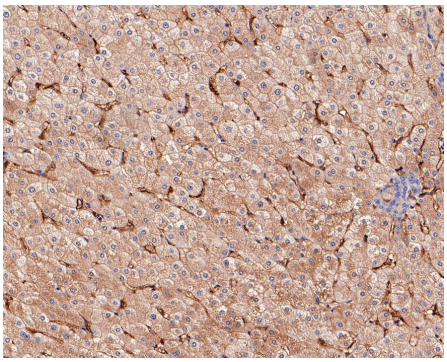

Fig9: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-CD14 antibody (HA750232) at 1/800 dilution.

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 (HA750232) at 1/800 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.

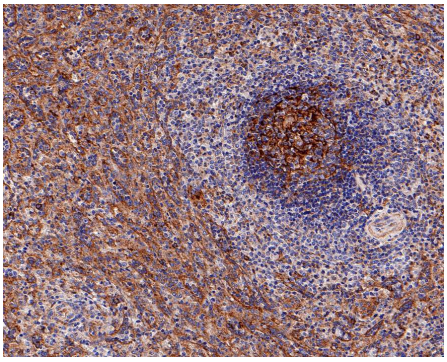

Fig10: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD14 antibody (HA750232) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750232) at 1/200 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.

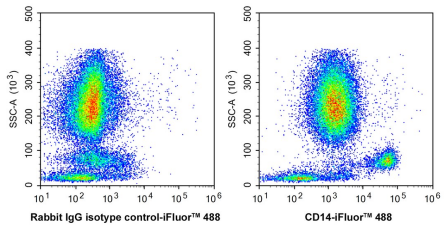

Fig11: Flow cytometric analysis of human peripheral blood cells labelling CD14 (HA750232).

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

Background References

1. Dutertre CA et al. Deciphering the stromal and hematopoietic cell network of the adventitia from non-aneurysmal and aneurysmal human aorta. PLoS One 9:e89983 (2014).
2. Hsu RY et al. LPS-induced TLR4 signaling in human colorectal cancer cells increases beta1 integrin-mediated cell adhesion and liver metastasis. Cancer Res 71:1989-98 (2011).