Anti-CD14 Antibody [PD01-63]

HA721321

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Tissue
Molecular Wt:	Predicted band size: 40 kDa
Clone number:	PD01-63

Description: CD14 is a 55-kDa protein found as a glycosylphosphatidylinositol (GPI)- anchored protein on the surface of monocytes, macrophages, and polymorphonuclear leukocytes, and as a soluble protein in the blood. Its main function is to serve as a receptor for lipopolysaccharide (LPS). 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. Besides its role in endotoxin signaling, it has been proposed that CD14 is involved in the transportation of other lipids, cell-cell interactions during different immune responses, and recognition of apoptotic cells. CD14 is highly expressed on the surface of monocytes/macrophages and strongly up-regulated during the differentiation of monocytic precursor cells into mature monocytes. Therefore, CD14 has been commonly used as a differentiation marker for monocytes/macrophages. An antibody to CD14 also labels Langerhans' cells and dendritic cells.

Immunogen: Synthetic peptide within human CD14 aa 300-375.

Positive control: NIH/3T3 cell lysate, SiHa cell lysate, human colon carcinoma tissue, human liver tissue, human tonsil tissue.

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

Database links: SwissProt: P08571 Human | P10810 Mouse

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200-1:1,000
IF-Tissue	1:200

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). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

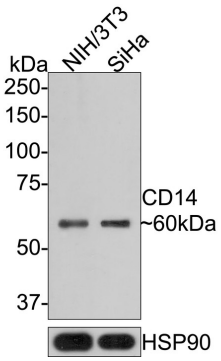

Fig1: Western blot analysis of CD14 on different lysates with Rabbit anti-CD14 antibody (HA721321) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: SiHa cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;

8% 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 (HA721321) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

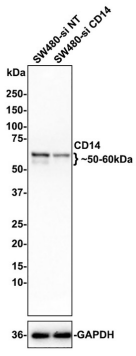

Fig2: Western blot analysis of CD14 on different lysates with Rabbit anti-CD14 antibody (HA721321) at 1/1,000 dilution.

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: 25 seconds;
ECL: merk

4-20% SDS-PAGE gel.

HA721321 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 (HA721321, 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

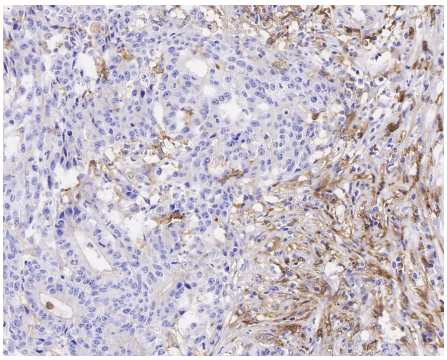

Fig3: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-CD14 antibody (HA721321) at 1/1,000 dilution.

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 (HA721321) 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.

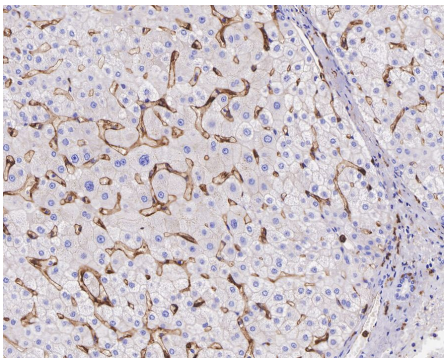

Fig4: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-CD14 antibody (HA721321) at 1/1,000 dilution.

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 (HA721321) 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.

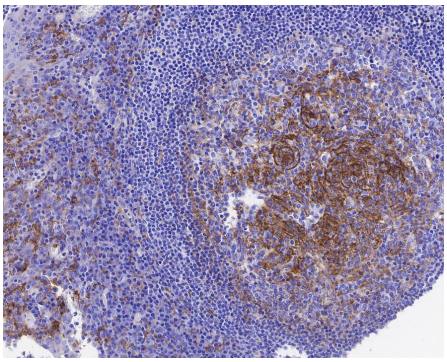

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD14 antibody (HA721321) at 1/200 dilution.

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 (HA721321) 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.

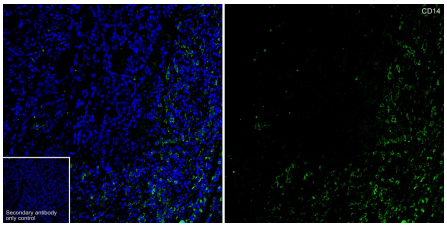

Fig6: Application: IF-Tissue

Species: Human

Site: colon cancer

Sample: Paraffin-embedded section

Antibody concentration: 1/200

Background References

1. Ciesielska A et al. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2021 Feb
2. Nakamizo S et al. Single-cell analysis of human skin identifies CD14+ type 3 dendritic cells co-producing IL1B and IL23A in psoriasis. Julia V, Hacini-Rachinel F, Kabashima K, Ginhoux F.
3. J Exp Med. 2021 Sep