

Anti-CD16 Antibody [PD00-12]

HA721149



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	IHC-P, IF-Tissue, IF-Cell, mIHC, WB
Molecular Wt:	Predicted band size: 29 kDa
Clone number:	PD00-12

Description: This gene encodes a receptor for the Fc portion of immunoglobulin G, and it is involved in the removal of antigen-antibody complexes from the circulation, as well as other responses, including antibody dependent cellular mediated cytotoxicity and antibody dependent enhancement of virus infections. This gene (FCGR3A) is highly similar to another nearby gene (FCGR3B) located on chromosome 1. The receptor encoded by this gene is expressed on natural killer (NK) cells as an integral membrane glycoprotein anchored through a transmembrane peptide, whereas FCGR3B is expressed on polymorphonuclear neutrophils (PMN) where the receptor is anchored through a phosphatidylinositol (PI) linkage. Mutations in this gene are associated with immunodeficiency 20, and have been linked to susceptibility to recurrent viral infections, susceptibility to systemic lupus erythematosus, and alloimmune neonatal neutropenia. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. CD16 (FcγRIII) is a low affinity Fc receptor that binds to IgG antibodies. Two forms of the receptor, CD16A (FcγRIIIa) and CD16B (FcγRIIIb) are present in natural killer cells, neutrophils, monocytes and activated macrophages. Antibody-dependent cell-mediated cytotoxicity (ADCC), cytokine release and microbe killing can be induced upon binding with CD16. Sconocchia, et al. demonstrated in a recent study that colorectal carcinoma patients with high CD16+ cell infiltration was associated with improved overall survival after adjusting for known prognostic factors and this association was independent from CD8+ lymphocyte infiltration and presence of metastases.

Immunogen:	Recombinant protein within human CD16 aa 17-208 (Extracellular).
Positive control:	Human spleen tissue, human liver carcinoma tissue, human muscle tissue, 293T, Jurkat, THP-1 treated with 100ng/mL TPA for 72 hours cell lysate.
Subcellular location:	Cell membrane, Secreted.
Database links:	SwissProt: P08637 Human
Recommended Dilutions:	
IHC-P	1:1,000
IF-Tissue	1:200
IF-Cell	1:100
mIHC	1:1,000
WB	1:5,000
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.
Purity:	Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

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Technical:0086-571-89986345

Service mail:support@huabio.cn

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Images

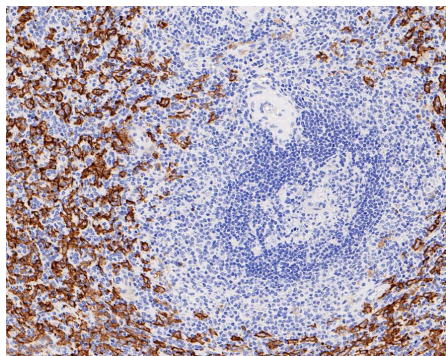


Fig1: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD16 antibody (HA721149) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA721149) 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.

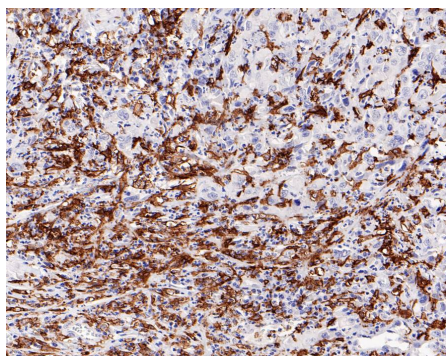


Fig2: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-CD16 antibody (HA721149) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA721149) 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.

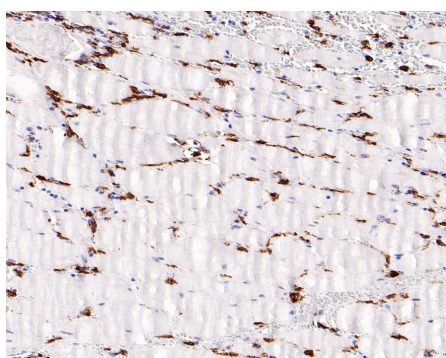


Fig3: Immunohistochemical analysis of paraffin-embedded human muscle tissue with Rabbit anti-CD16 antibody (HA721149) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA721149) 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.

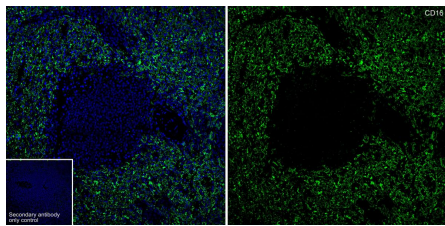


Fig4: Immunofluorescence analysis of paraffin-embedded human spleen tissue labeling CD16 with Rabbit anti-CD16 antibody (HA721149) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721149, green) at 1/200 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

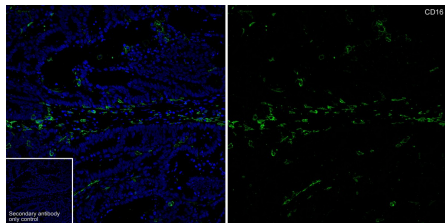


Fig5: Immunofluorescence analysis of paraffin-embedded human colon cancer tissue labeling CD16 with Rabbit anti-CD16 antibody (HA721149) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721149, green) at 1/200 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

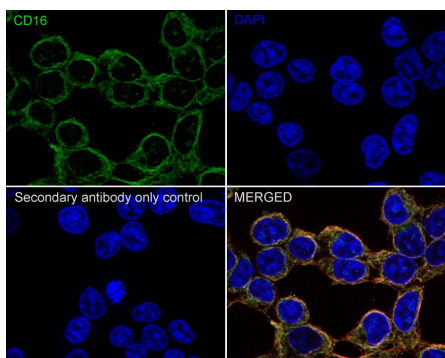
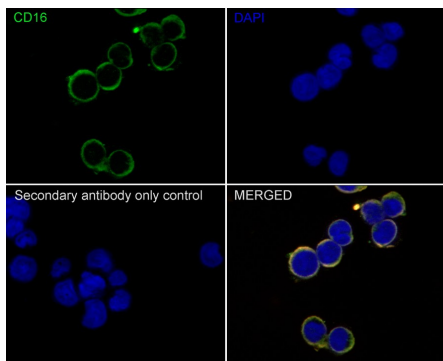


Fig6: Immunocytochemistry analysis of 293T cells labeling CD16 with Rabbit anti-CD16 antibody (HA721149) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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-CD16 antibody (HA721149) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig7: Immunocytochemistry analysis of Jurkat cells labeling CD16 with Rabbit anti-CD16 antibody (HA721149) at 1/100 dilution.



Cells were fixed in 80% precooled methanol 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-CD16 antibody (HA721149) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

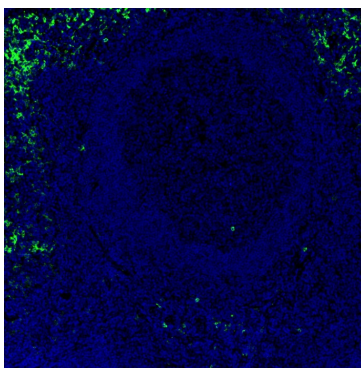
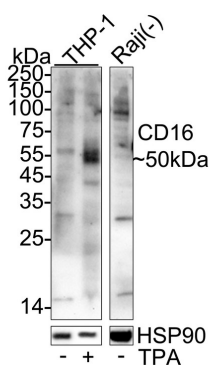


Fig8: mlHC analysis of human tonsils tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-CD16 antibody (HA721149) at 1/1,000 dilution. The immunostaining was performed with the IRISKitCmTSA Kit (900808). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Fig9: Western blot analysis of CD16 on different lysates with Rabbit anti-CD16 antibody (HA721149) at 1/5,000 dilution.



Lane 1: THP-1 cell lysate (20 µg/Lane)
 Lane 2: THP-1 treated with 100ng/mL TPA for 72 hours cell lysate (20 µg/Lane)
 Lane 3: Raji cell lysate (negative) (20 µg/Lane)

Predicted band size: 29 kDa
 Observed band size: 50 kDa

Exposure time: 2 minutes 30 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA721149) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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

Background References

1. Cheng ZF. et. al. A novel endogenous CD16-Expressing Natural Killer Cell for cancer immunotherapy. Biochem Biophys Rep. 2021 Feb
2. Zhang J. et. al. The immune potential of decidua-resident CD16(+)CD56(+) NK cells in human pregnancy. Hum Immunol. 2021 May

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