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Anti-CD59 Antibody Picoband™(monoclonal, 3C10)

Catalog # ABO14916

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P13987
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-CD59 Antibody Picoband™ (monoclonal, 3C10) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	966
Other Names	CD59 glycoprotein, 1F5 antigen, 20 kDa homologous restriction factor, HRF-20, HRF20, MAC-inhibitory protein, MAC-IP, MEM43 antigen, Membrane attack complex inhibition factor, MACIF, Membrane inhibitor of reactive lysis, MIRL, Protectin, CD59, CD59, MIC11, MIN1, MIN2, MIN3, MSK21
Calculated MW	14177
Application Details	Western blot, 0.1-0.5 □g/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6, Human
Source	Eukaryota
Protein Name	CD59 molecule, complement regulatory protein
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 3C10
Immunogen	E.coli-derived human CD59 recombinant protein (Position: L26-N102). Human CD59 shares 47.1% amino acid (aa) sequence identity with rat CD59.
Purification	Immunogen affinity purified.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C

for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	CD59 {ECO:0000303|PubMed:2475570, ECO:0000312|HGNC:HGNC:1689}
Function	Potent inhibitor of the complement membrane attack complex (MAC) action, which protects human cells from damage during complement activation (PubMed:11882685, PubMed:1698710, PubMed:2475111, PubMed:2475570, PubMed:2606909, PubMed:9053451). Acts by binding to the beta-haipins of C8 (C8A and C8B) components of the assembling MAC, forming an intermolecular beta-sheet that prevents incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore (PubMed:11882685, PubMed:1698710, PubMed:36797260).
Cellular Location	Cell membrane; Lipid-anchor, GPI-anchor. Secreted. Note=Localizes to the cell surface (PubMed:36797260). Soluble form found in a number of tissues (PubMed:8670172).

Background

This gene encodes a cell surface glycoprotein that regulates complement-mediated cell lysis, and it is involved in lymphocyte signal transduction. And this protein is a potent inhibitor of the complement membrane attack complex, whereby it binds complement C8 and/or C9 during the assembly of this complex, thereby inhibiting the incorporation of multiple copies of C9 into the complex, which is necessary for osmolytic pore formation. It also plays a role in signal transduction pathways in the activation of T cells. Mutations in this gene cause CD59 deficiency, a disease resulting in hemolytic anemia and thrombosis, and which causes cerebral infarction. Multiple alternatively spliced transcript variants, which encode the same protein, have been identified for this gene.

Images

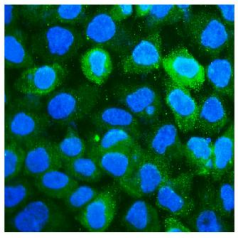
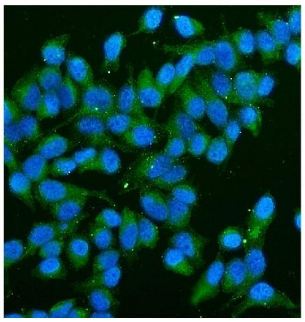


Figure 2. IF analysis of CD59 using anti-CD59 antibody (M00914-2).
CD59 was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-CD59 Antibody (M00914-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Figure 3. IF analysis of CD59 using anti-CD59 antibody (M00914-2).
CD59 was detected in immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-CD59 Antibody (M00914-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary



antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

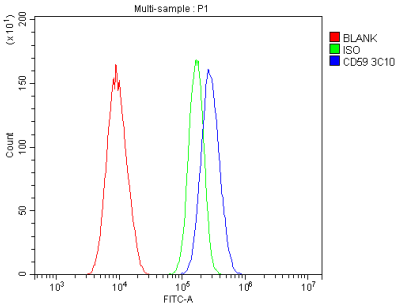


Figure 4. Flow Cytometry analysis of A549 cells using anti-CD59 antibody (M00914-2).
Overlay histogram showing A549 cells stained with M00914-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CD59 Antibody (M00914-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

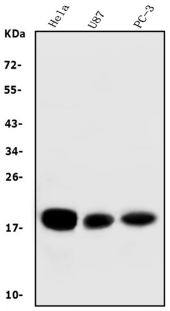


Figure 1. Western blot analysis of CD59 using anti-CD59 antibody (M00914-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates;
Lane 2: human U-87MG whole cell lysates;
Lane 3: human PC-3 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-CD59 antigen affinity purified monoclonal antibody (Catalog # M00914-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for CD59 at approximately 19KD. The expected band size for CD59 is at 19KD.

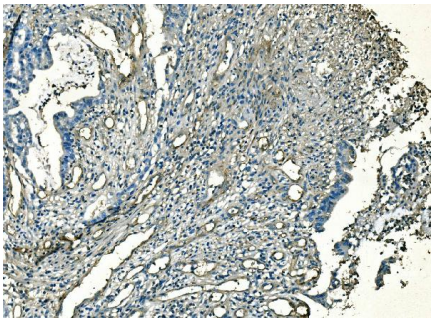


Figure 5. IHC analysis of CD59 using anti-CD59 antibody (M00914-2).
CD59 was detected in paraffin-embedded section of human colorectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CD59 Antibody (M00914-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30

minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.