Anti-SIRP alpha Antibody [A3E3]

EM1902-37



Product Type: Mouse monoclonal IgG1, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 55 kDa.

Clone number: A3E3

Description: Immunoglobulin-like cell surface receptor for CD47. Acts as docking protein and induces

translocation of PTPN6, PTPN11 and other binding partners from the cytosol to the plasma membrane. Supports adhesion of cerebellar neurons, neurite outgrowth and glial cell attachment. May play a key role in intracellular signaling during synaptogenesis and in synaptic function (By similarity). Involved in the negative regulation of receptor tyrosine kinase-coupled cellular responses induced by cell adhesion, growth factors or insulin. Mediates negative regulation of phagocytosis, mast cell activation and dendritic cell activation. CD47 binding prevents maturation of immature dendritic cells and inhibits cytokine

production by mature dendritic cells.

Immunogen: Recombinant protein within human SIRP alpha aa 1-400.

Positive control: SW480 cell lysates, THP-1, human lung cancer tissue.

Subcellular location: Membrane.

Database links: SwissProt: P78324 Human

Recommended Dilutions:

WB 1:500 IF-Cell 1:50 IHC-P 1:50-1:200 FC 1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Images

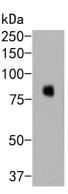


Fig1: Western blot analysis of SIRP alpha on SW480 cell lysate. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (EM1902-37, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:5,000 dilution was used for 1 hour at room temperature.

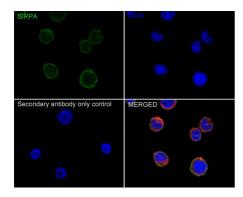


Fig2: Immunocytochemistry analysis of THP-1 cells labeling SIRP alpha with Mouse anti-SIRP alpha antibody (EM1902-37) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 Mouse anti-SIRP alpha antibody (EM1902-37) at 1/50 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

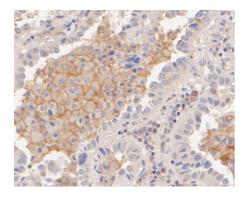


Fig3: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue using anti-SIRP alpha antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (EM1902-37, 1/100) for 30 minutes 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.

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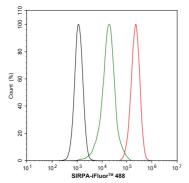


Fig4: Flow cytometric analysis of THP-1 cells labeling SIRP alpha.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (EM1902-37, 1/1,000) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for 30 minutes, the cells were stained with a iFluor 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. 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. Timms J.F.et.al.SHPS-1 is a scaffold for assembling distinct adhesion-regulated multi-protein complexes in macrophages.Curr. Biol. 9:927-930(1999).