

Anti-Myc tag Antibody [PSH17-81]

HA723950



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Species independent
Applications:	WB, IF-Cell, IHC-P, FC, IP
Clone number:	PSH17-81

Description: A myc tag is a polypeptide protein tag derived from the c-myc gene product that can be added to a protein using recombinant DNA technology. It can be used for affinity chromatography, then used to separate recombinant, overexpressed protein from wild type protein expressed by the host organism. It can also be used in the isolation of protein complexes with multiple subunits. A myc tag can be used in many different assays that require recognition by an antibody. If there is no antibody against the studied protein, adding a myc-tag allows one to follow the protein with an antibody against the Myc epitope. Examples are cellulite localization studies by immunofluorescence or detection by Western blotting. The peptide sequence of the myc-tag is (in 1- and 3-letter codes, respectively): EQKLISEEDL and Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu. The tag is approximately 1202 Daltons in atomic mass and has 10 amino acids. It can be fused to the C-terminus and the N-terminus of a protein. It is advisable not to fuse the tag directly behind the signal peptide of a secretory protein, since it can interfere with translocation into the secretory pathway. A monoclonal antibody against the myc epitope, named 9E10, is available from the non-commercial Developmental Studies Hybridoma Bank.

Immunogen: Synthetic peptide EQKLISEEDL conjugated to KLH.

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:15,000
IHC-P	1:30,000
FC	1:5,000
IP	1-2µg/sample

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.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

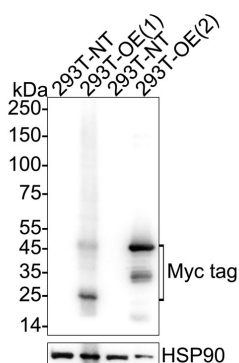


Fig1: Western blot analysis of Myc tag on different lysates with Rabbit anti-Myc tag antibody (HA723950) at 1/20,000 dilution.

Lane 1: 293T transfected with Myc-tagged empty control cell lysate

Lane 2: 293T transfected with Myc-tagged Claudin18.2 (C-terminal) cell lysate

Lane 3: 293T transfected with Myc-tagged empty control cell lysate

Lane 4: 293T transfected with Myc-tagged ACAT2 (C-terminal) cell lysate

Lysates/proteins at 10 µg/Lane.

Exposure time: 4 seconds; ECL: K1801;

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 (HA723950) at 1/20,000 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.

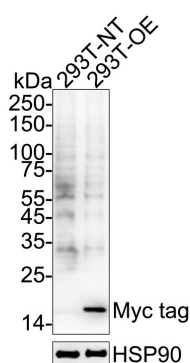


Fig2: Western blot analysis of Myc tag on different lysates with Rabbit anti-Myc tag antibody (HA723950) at 1/5,000 dilution.

Lane 1: 293T transfected with Myc-tagged empty control cell lysate

Lane 2: 293T transfected with Myc-tagged Histone H3.1 (N-terminal) cell lysate

Lysates/proteins at 10 µg/Lane.

Exposure time: 40 seconds; ECL: K1802;

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 (HA723950) 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.

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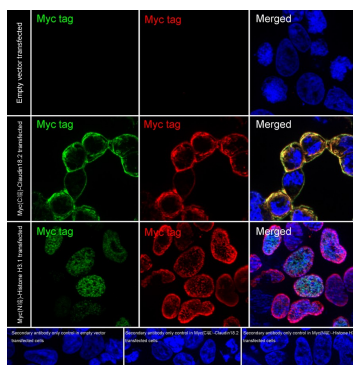
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Fig3: Immunocytochemistry analysis of 293T cells labeling Myc tag with Rabbit anti-Myc tag antibody (HA723950) at 1/15,000 dilution.



293T cells, transfected with Myc-tagged empty control (top, negative) / Myc-tagged Claudin18.2 (C-terminal) (middle, positive) / Myc-tagged Histone H3.1 (N-terminal) (bottom, positive) expression vector, respectively, 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 Rabbit anti-Myc tag antibody (HA723950) at 1/15,000 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.

Myc tag (HA601081, red) was stained at 1/1,000 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.

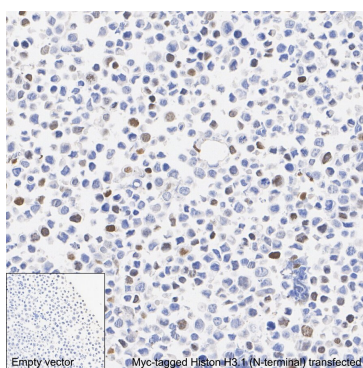
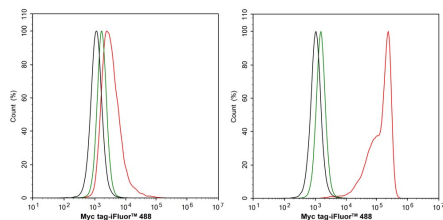


Fig4: Immunohistochemical analysis of paraffin-embedded 293T cells transfected with Myc-tagged Histone H3.1 (N-terminal) with Rabbit anti-Myc tag antibody (HA723950) at 1/30,000 dilution.

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 (HA723950) at 1/30,000 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: Flow cytometric analysis of 293T cells transfected with Myc-tagged empty control (left) / 293T cells transfected with Myc-tagged ACAT2 (C-terminal) (right) labeling Myc tag.



Cells were fixed and permeabilized. Then stained with the primary antibody (HA723950, 1/5,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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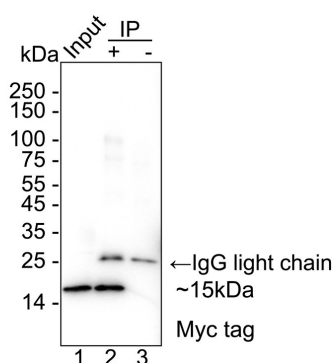


Fig6: Myc tag was immunoprecipitated from 0.2 mg 293T transfected with Myc-tagged Histone H3.1 (N-terminal) cell lysate with HA723950 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using HA723950 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 293T transfected with Myc-tagged Histone H3.1 (N-terminal) cell lysate (input)

Lane 2: HA723950 IP in 293T transfected with Myc-tagged Histone H3.1 (N-terminal) cell lysate

Lane 3: Rabbit IgG instead of HA723950 in 293T transfected with Myc-tagged Histone H3.1 (N-terminal) cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)

Exposure time: 42 seconds; ECL: K1801

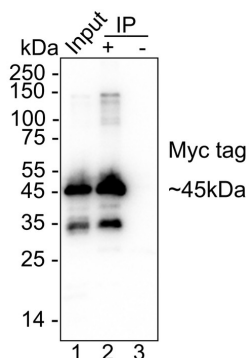


Fig7: Myc tag was immunoprecipitated from 0.2 mg 293T transfected with Myc-tagged ACAT2 (C-terminal) cell lysate with HA723950 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using HA723950 at 1/20,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 293T transfected with Myc-tagged ACAT2 (C-terminal) cell lysate (input)

Lane 2: HA723950 IP in 293T transfected with Myc-tagged ACAT2 (C-terminal) cell lysate

Lane 3: Rabbit IgG instead of HA723950 in 293T transfected with Myc-tagged ACAT2 (C-terminal) cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)

Exposure time: 2 seconds; ECL: K1801

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

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