Anti-Myc tag Antibody [A3C8-R-2] - BSA and Azide free

HA610018

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Species independent
Applications:	ELISA, WB, IP, IF-Cell
Clone number:	A3C8-R-2

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 keyhole limpet haemocyanin.

Recommended Dilutions:

ELISA	1:10,000
WB	1:100,000-1:200,000
IP	Use at an assay dependent concentration.
IF-Cell	1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

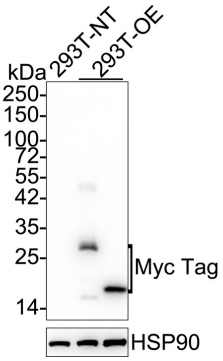

Fig1: Western blot analysis of Myc tag on different lysates with Mouse anti-Myc tag antibody (HA610018) at 1/100,000 dilution.

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 Histone H3.1 (N-terminal) cell lysate

Lysates/proteins at 10 μg/Lane.

Exposure time: 1 minute 2 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 (HA610018) at 1/100,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

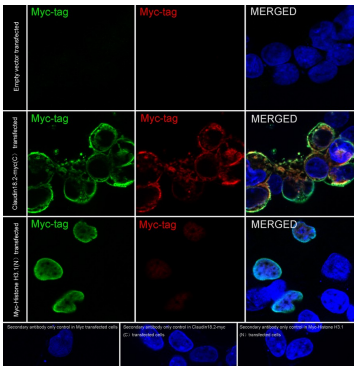

Fig2: Immunocytochemistry analysis of 293T cells labeling Myc tag with Mouse anti-Myc tag antibody (HA610018) at 1/1,000 dilution.

293T cells, transfected with Myc-tagged empty control, Claudin18.2 (C-terminal) or Histone H3.1 (N-terminal) expression vector, respectively, were fixed in 4% paraformaldehyde for 10 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-Myc tag antibody (HA610018) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Myc Tag (R1208-1, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibody at 1/1,000 dilution.

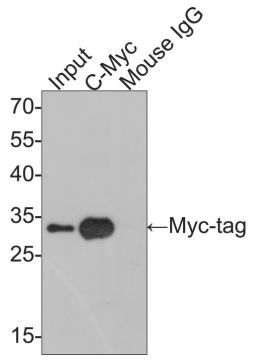

Fig3: Myc-tag was immunoprecipitated in 2µg C-terminal Myc-tag fusion protein lysate with HA610018. Western blot was performed from the immunoprecipitate using HA610018 at 1/2,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1:5,000 dilution was used for 60 mins at room temperature.

Lane 1: C-terminal Myc-tag fusion protein lysate (input).
Lane 2: HA610018 IP in C-terminal Myc-tag fusion protein lysate.
Lane 3: Mouse IgG instead of HA610018 IP in C-terminal Myc-tag fusion protein lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

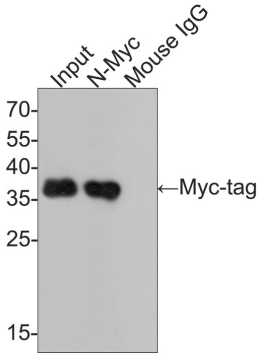

Fig4: Myc-tag was immunoprecipitated in 2µg N-terminal Myc-tag fusion protein lysate with HA610018. Western blot was performed from the immunoprecipitate using HA610018 at 1/500 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1:2,000 dilution was used for 60 mins at room temperature.

Lane 1: N-terminal Myc-tag fusion protein lysate (input).
Lane 2: HA610018 IP in N-terminal Myc-tag fusion protein lysate.
Lane 3: Mouse IgG instead of HA610018 IP in N-terminal Myc-tag fusion protein lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

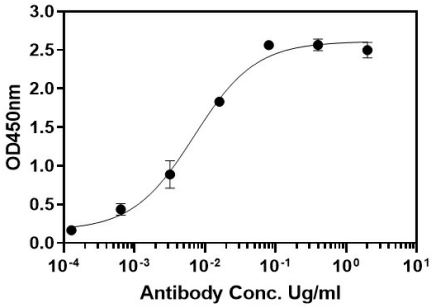

Fig5: Myc tag Antibody (HA610018) in indirect ELISA.

Indirect ELISA analysis of Myc tag was performed by coating wells of a 96-well plate with 50 µl per well of Myc tag antigen diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with StartingBlock blocking buffer, and incubated with 50 µl per well of a mouse Myc tag monoclonal antibody starting at a concentration of 20 µg/mL and serially diluting it to a concentration of 1.28 ng/mL for 1 hour at 37℃. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Mouse IgG secondary antibody at a dilution of 1:10,000 for 30 minutes at 37℃. Detection was performed using an Ultra TMB Substrate for 5 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.