

Anti-DYKDDDDK Tag (FLAG) Antibody [A2-A4-R] - BSA and Azide free

HA610054



Product Type: Recombinant Mouse monoclonal IgG1, primary antibodies

Species reactivity: Species independent

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

Clone number: A2-A4-R

Description: FLAG-tag, or FLAG octapeptide, or FLAG epitope, is a polypeptide protein tag that can be added to a protein using recombinant DNA technology, having the sequence motif DYKDDDDK (where D=aspartic acid, Y=tyrosine, and K=lysine). It is one of the most specific tags and it is an artificial antigen to which specific, high affinity monoclonal antibodies have been developed and hence can be used for protein purification by affinity chromatography and also can be used for locating proteins within living cells. It has been 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, because its mild purification procedure tends not to disrupt such complexes. It has been used to obtain proteins of sufficient purity and quality to carry out 3D structure determination by x-ray crystallography. A FLAG-tag can be used in many different assays that require recognition by an antibody. If there is no antibody against a given protein, adding a FLAG-tag to a protein allows the protein to be studied with an antibody against the FLAG sequence. Examples are cellular localization studies by immunofluorescence, immunoprecipitation or detection by SDS PAGE protein electrophoresis and Western blotting. The peptide sequence of the FLAG-tag from the N-terminus to the C-terminus is: DYKDDDDK (1012 Da). Additionally, it may be used in tandem, commonly the 3xFLAG peptide: DYKDHD-G-DYKDHD-I-DYKDDDDK (with the final tag encoding an enterokinase cleavage site). It can be fused to the C-terminus or the N-terminus of a protein, or inserted within a protein. The tyrosine residue in the FLAG-tag can be sulfated, which can affect antibody recognition of the FLAG epitope. The FLAG-tag can be used in conjunction with other affinity tags, for example a polyhistidine tag (His-tag), HA-tag or myc-tag.

Immunogen: Synthetic peptide immune sequence is N-DYKDDDDK-C.

Recommended Dilutions:

WB 1:5,000-1:10,000

IP 1-2 μ g/sample

IF-Cell 1:1,000-1:5,000

IHC-P 1:1,000

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co.,Ltd.

Orders:0086-571-88062880

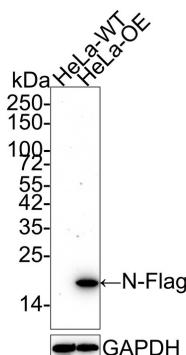
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Images

Fig1: Western blot analysis of DYKDDDDK Tag (FLAG) on different lysates with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/5,000 dilution.



Lane 1: HeLa cell lysate

Lane 2: HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cell lysate

Lysates/proteins at 5 µg/Lane.

Predicted band size: 15 kDa

Observed band size: 18 kDa

Exposure time: 1 minute 30 seconds;

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 (HA610054) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of DYKDDDDK Tag (FLAG) on different lysates with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/5,000 dilution.

Lane 1: L-929 cell lysate

Lane 2: L-929 transfected with FLAG-tagged CD5 (C-terminal) cell lysate

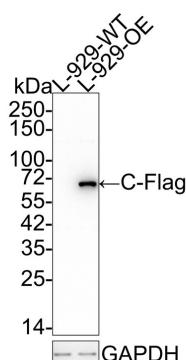
Lysates/proteins at 5 µg/Lane.

Predicted band size: 65 kDa

Observed band size: 65 kDa

Exposure time: 1 minute 59 seconds;

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 (HA610054) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

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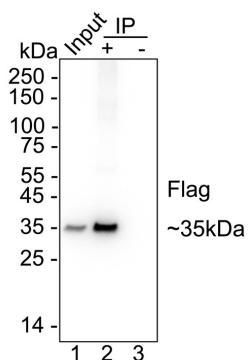


Fig3: DYKDDDDK Tag (FLAG) was immunoprecipitated from 0.2 mg 293T transfected with FLAG-tagged PD-L1 (C-terminal) cell lysate with HA610054 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using HA610054 at 1/20,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 293T transfected with FLAG-tagged PD-L1 (C-terminal) cell lysate (input)
 Lane 2: HA610054 IP in 293T transfected with FLAG-tagged PD-L1 (C-terminal) cell lysate
 Lane 3: Mouse IgG instead of HA610054 in 293T transfected with FLAG-tagged PD-L1 (C-terminal) cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
 Exposure time: 2 seconds; ECL: K1801

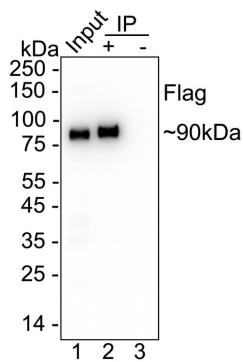


Fig4: DYKDDDDK Tag (FLAG) was immunoprecipitated from 0.2 mg 293T transfected with FLAG-tagged MYRF (N-terminal) cell lysate with HA610054 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using HA610054 at 1/20,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 293T transfected with FLAG-tagged MYRF (N-terminal) cell lysate (input)
 Lane 2: HA610054 IP in 293T transfected with FLAG-tagged MYRF (N-terminal) cell lysate
 Lane 3: Mouse IgG instead of HA610054 in 293T transfected with FLAG-tagged MYRF (N-terminal) cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
 Exposure time: 2 seconds; ECL: K1801

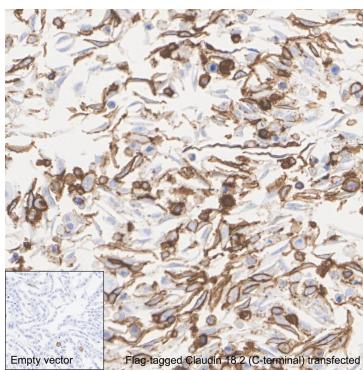


Fig5: Immunohistochemical analysis of paraffin-embedded HeLa transfected with FLAG-tagged Claudin 18.2 (C-terminal) cells with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/1,000 dilution.

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 (HA610054) 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.

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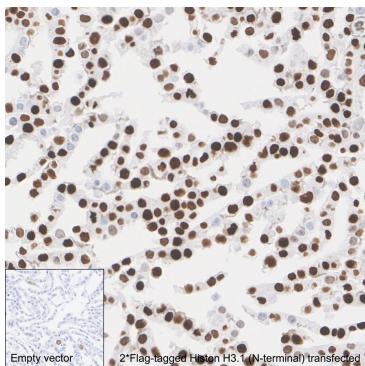
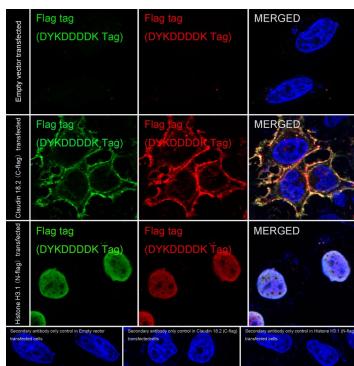


Fig6: Immunohistochemical analysis of paraffin-embedded HeLa transfected with 2*FLAG-tagged Histone H3.1 (N-terminal) cells with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/1,000 dilution.

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 (HA610054) 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.

Fig7: Immunocytochemistry analysis of HeLa cells labeling DYKDDDDK Tag (FLAG) with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/1,000 dilution.



HeLa cells, transfected with empty control (top, negative) / Flag-tagged Claudin 18.2 (C-terminal) (middle, positive) / 2*Flag-tagged Histon 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 Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA610054) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) 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.

DYKDDDDK Tag (FLAG) (HA724169, green) was stained at 1/1,000 dilution overnight at +4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution.

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

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