Anti-DYKDDDDK Tag (FLAG) Antibody [A2-A4-R] HA601167

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	2-5 μg/ml.
IF-Cell	1:250-1:10,000
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

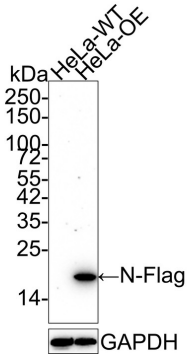

Fig1: Western blot analysis of DYKDDDDK Tag (FLAG) on different lysates with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/2,000 dilution.

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 (HA601167) at 1/2,000 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.

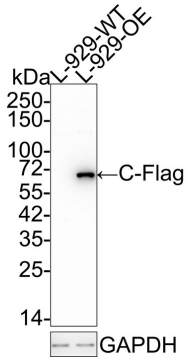

Fig2: Western blot analysis of DYKDDDDK Tag (FLAG) on different lysates with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/2,000 dilution.

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 (HA601167) at 1/2,000 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.

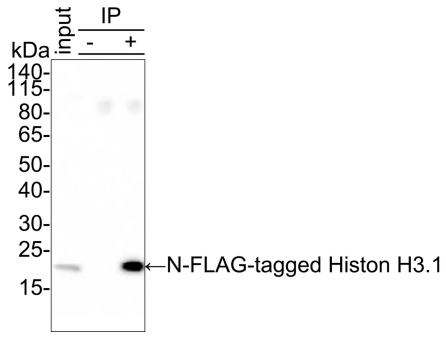

Fig3: DYKDDDDK Tag (FLAG) was immunoprecipitated in 2µg HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cell lysate with HA601167. Western blot was performed from the immunoprecipitate using DYKDDDDK Tag(FLAG) (M1403-2) at 1/1,000 dilution. Anti-Mouse IgG for IP Nano-Secondary Antibody (NBI02H) at 1:5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cell lysate (input).
Lane 2: Mouse IgG instead of HA601167 IP in HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cell lysate.
Lane 3: HA601167 IP in HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

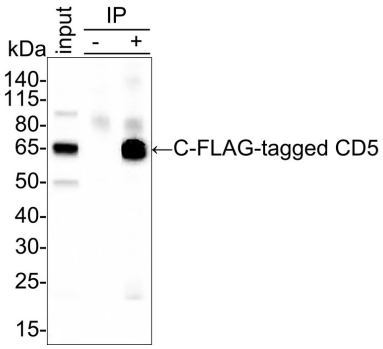

Fig4: DYKDDDDK Tag (FLAG) was immunoprecipitated in 2µg L-929 transfected with FLAG-tagged CD5 (C-terminal) cell lysate with HA601167. Western blot was performed from the immunoprecipitate using DYKDDDDK Tag(FLAG) (M1403-2) at 1/1,000 dilution. Anti-Mouse IgG for IP Nano-Secondary Antibody (NBI02H) at 1:5,000 dilution was used for 1 hour at room temperature.

Lane 1: L-929 transfected with FLAG-tagged CD5 (C-terminal) cell lysate (input).
Lane 2: Mouse IgG instead of HA601167 IP in L-929 transfected with FLAG-tagged CD5 (C-terminal) cell lysate.
Lane 3: HA601167 IP in L-929 transfected with FLAG-tagged CD5 (C-terminal) cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

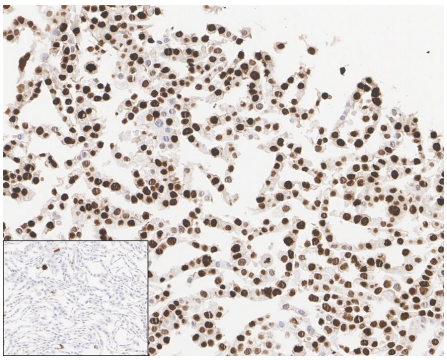

Fig5: Immunohistochemical analysis of paraffin-embedded HeLa transfected with FLAG-tagged Histon H3.1 (N-terminal) cells with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA601167) 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.

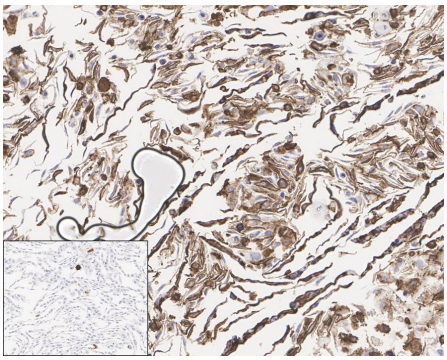

Fig6: Immunohistochemical analysis of paraffin-embedded HeLa transfected with FLAG-tagged Claudin 18.2 (C-terminal) cells with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA601167) 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.

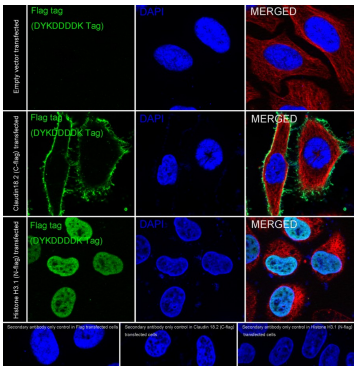

Fig7: Immunocytochemistry analysis of HeLa cells labeling DYKDDDDK Tag (FLAG) with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/250 dilution.

HeLa cells, transfected with FLAG-tagged empty control, Claudin 18.2 (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-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/250 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.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

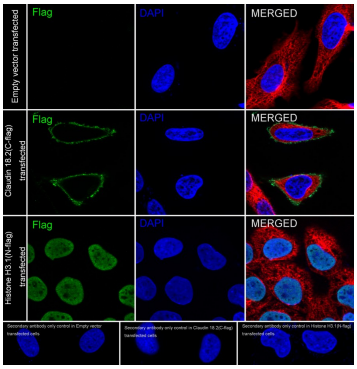

Fig8: Immunocytochemistry analysis of HeLa cells labeling DYKDDDDK Tag (FLAG) with Mouse anti-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/10,000 dilution.

HeLa cells, transfected with FLAG-tagged empty control, Claudin 18.2 (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-DYKDDDDK Tag (FLAG) antibody (HA601167) at 1/10,000 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.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.