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

# M1403-2

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| **Product Type:** | Mouse monoclonal IgG1, primary antibodies |
| **Species reactivity:** | Species independent |
| **Applications:** | WB, IP, ELISA, IF-Cell |
| **Clone number:** | A2-A4 |

**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.

**Positive control:** DYKDDDDK Tag recombinant protein, C-terminal FLAG-tagged recombinant protein, N-terminal FLAG-tagged recombinant protein.

**Recommended Dilutions:**

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| **WB** | 1:20,000-1:50,000 |
| **IP** | 2-5 μg/ml. |
| **IF-Cell** | 1:500-1:10,000 |

**Storage Buffer:** 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

**Purity:** Protein G affinity purified.

## Images

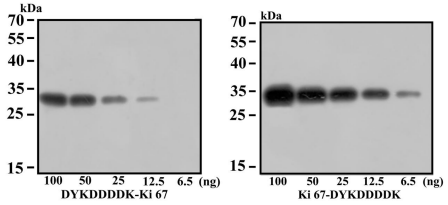


**Fig1:** Western blot analysis of DYKDDDDK Tag on Flag-tagged recombinant protein Flag-Ki67 and Ki67-Flag at different cell lysates level.

Predicted band size: 35 kDa;
Observed band size: 35 kDa;
Exposure time: 60 seconds;
SDS-PAGE gel concentration:12%

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (M1403-2, 1/20,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/200,000 dilution was used for 1 hour at room temperature.

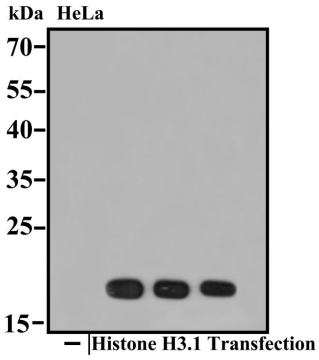


**Fig2:** Western blot analysis of DYKDDDDK Tag on Flag-tagged recombinant protein 2xflag-Histone H3.1 at different dilutions of HeLa cells with anti-DYDDDDK antibody (M1403-2).

Lane 1: Non-transfected HeLa cell lysates (1/2000)
Lane 2~4: Transfected HeLa cell lysates (1/2500; 1/5000; 1/10,000)
Predicted band size: 15 kDa;
Observed band size: 18 kDa;
Exposure time: 30 seconds;
SDS-PAGE gel concentration:12%.

Cell lysate at 10ug per lane.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (M1403-2) was used at different dilutions in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/200,000 dilution was used for 1 hour at room temperature.

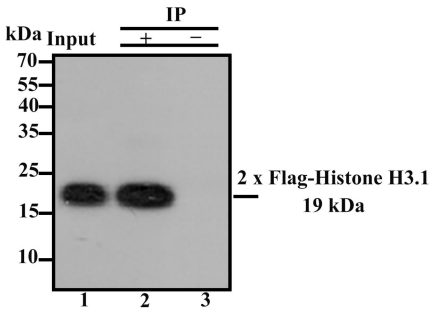


**Fig3:** Immunoprecipitation analysis of N-Flag in HeLa cells.

Lane 1: HeLa transfected with DYKDDDDK-tagged human Histone H3.1 expression vector whole cell lysate (input).
Lane 2: M1403-2 IP in HeLa transfected with DDDDK-tagged human Histone H3.1 expression vector whole cell lysate.
Lane 3: Mouse IgG (HA1027) instead of M1403-2 in HeLa transfected with DYKDDDDK-tagged human Histone H3.1 expression vector whole cell lysate.

Predicted band size: 19 kDa;
Observed band size: 19 kDa;
Exposure time: 60 seconds;

SDS-PAGE gel concerntration: 15%.

DYKDDDDK Tag was immunoprecipitated from 0.5 mg Hela transfected with human 2x Flag- Histone H3.1 expression vector whole cell lysate with M1403-2 2 ug/mL. Western blot was performed from the immunoprecipitate using M1403-2 at 1/5,000 dilution for 1 hour at room temperature. The secondary antibody for IP detection reagent, Goat anti-Mouse IgG-HRP antibody (HA1006), was used at 1:100,000 dilution.

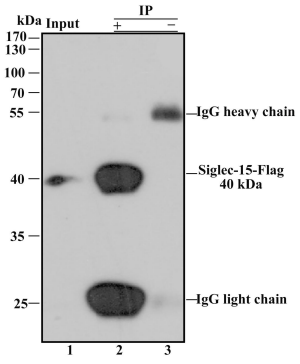


**Fig4:** Immunoprecipitation analysis of C-Flag in HeLa cells.

Lane 1: HeLa transfected with DYKDDDDK-tagged human Siglec15 expression vector whole cell lysate (input).
Lane 2: M1403-2 IP in HeLa transfected with DDDDK-tagged human Siglec15 expression vector whole cell lysate.
Lane 3: Mouse IgG (HA1027) instead of M1403-2 in HeLa transfected with DYKDDDDK-tagged human Siglec15 expression vector whole cell lysate.

DYKDDDDK Tag was immunoprecipitated from 0.5 mg Hela transfected with human Siglec15 expression vector whole cell lysate with M1403-2 2 ug/mL. Western blot was performed from the immunoprecipitate using M1403-2 at 1/2,000 dilution for 1 hour at room temperature. The secondary antibody for IP detection reagent, Goat anti-Mouse IgG-HRP antibody (HA1006), was used at 1:100,000 dilution.

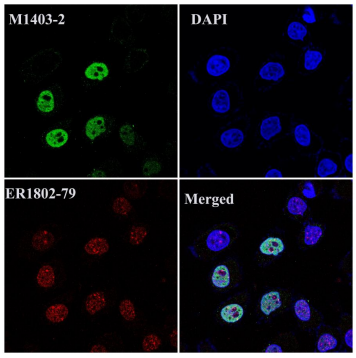


**Fig5:** Immunofluorescent analysis of 2xFlag tagged Histone H3.1 in HeLa cells.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling DYKDDDDK tag with M1403-2 at 1/500 dilution, followed by iFluor™ 488 Goat anti-mouse IgG antibody (HA1125) at 1/1000 dilution (green).

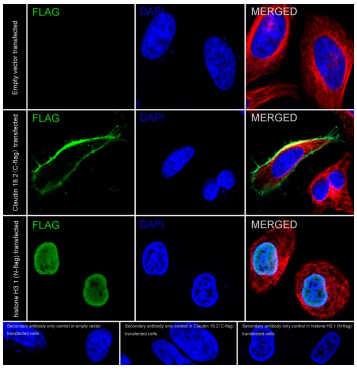


**Fig6:** Immunocytochemistry analysis of HeLa cells labeling DYKDDDDK Tag (FLAG) with Mouse anti-DYKDDDDK Tag (FLAG) antibody (M1403-2) at 1/10,000 dilution.

HeLa cells, transfected with empty control (top, negative) / Flag-tagged Claudin 18.2 (middle, positive) / Flag-tagged Histone H3.1 (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 (M1403-2) 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.

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