

#M20001

His-Tag (2A8) Mouse mAb

□ 100 µl (50 Western mini-blot)

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BACKGROUND

Plasmid vectors for the expression of coding regions of eukaryotic genes in bacterial, insect and mammalian hosts are in common usage; such expression vectors are frequently used to encode hybrid fusion proteins consisting of a eukaryotic target protein and a specialized region designed to aid in the purification and visualization of the target protein. A system that has proven to be very successful relies on the insertion of a six histidine (His6) sequence in the N-terminus of the encoded protein, allowing for efficient coupling to Ni²⁺-chelating resins and purification by single step affinity chromatography. This polyhistidine sequence can then be removed by specific cleavage at sites recognized by enzymes such as thrombin or enterokinase, permitting the separation of the target protein from the polyhistidine tag. Visualization of such fusion proteins can be achieved by utilizing antibodies generated against specific peptide sequences downstream from the multiple cloning site.

REFERENCES

1. Maniatis, T., et al. 1982. Molecular Cloning. Cold Spring Laboratory, Cold Spring Harbor, NY.
2. Hochuli, E. 1988. Large-scale chromatography of recombinant proteins. J. Chromatog. 444: 293-302.

SOURCE

This monoclonal antibody is produced by immunizing mice with a 6× His synthetic peptide (KLH-coupled).

SPECIFICITY

His-Tag (2A8) Mouse mAb detects over-expressed or recombinant proteins containing the 6× His epitope tag.

STORAGE

Store at -20°C. Stable for one year from the date of shipment.

REACTIVITY

All

ISOTYPE

Mouse IgG1

IMPORTANT

Use an **anti-MOUSE** secondary antibody to detect the 2A8 antibody.

RECOMMENDED ANTIBODY DILUTIONS

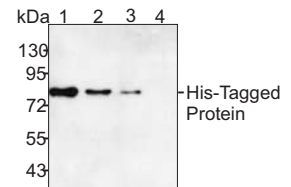
Western blotting	1:5000
Immunofluorescence	1:2000
Immunoprecipitation	1:100
ELISA	1:2000

*** For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1× TBS, 0.05% Tween-20 at 4°C with gentle shaking, overnight.**

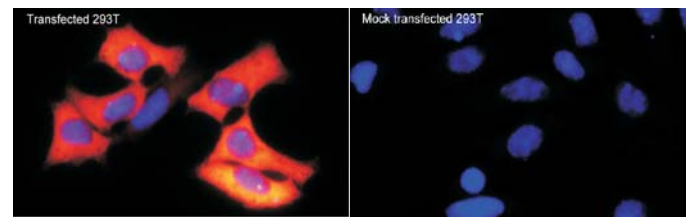
APPLICATION DATA



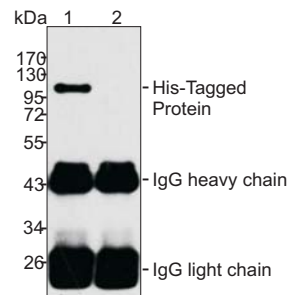
Western blot analysis of over-expressed His-tagged protein in 293T cell lysate, using His-tag (2A8) Mouse mAb. The antibody dilutions are 1:2000 (lane 1), 1:5000 (lane 2) and 1:10000 (lane 3). Each



2A8 can specifically detect 1 ng of recombinant protein. There is 6 ng, 3 ng, 1 ng, and 0 ng His-tagged recombinant protein in lanes 1-4, respectively, added with 20 µg of 293T cell lysate.



IF analysis of 293T cells transfected (left) with a His-tagged protein and a mock transfection (right, using the same protein without the His tag), using His-Tag (2A8) Mouse mAb at a 1:2000 dilution.



IP of extracts from 293T cells transfected (lane 1) with a His-tagged protein and a mock transfection (lane 2, using the same protein without the His tag), using His-Tag (2A8) Mouse mAb and probed on Western blot using the

COMPANION PRODUCTS

- #M20002 Myc-Tag (19C2) Mouse mAb
- #M20003 HA-Tag (26D11) Mouse mAb
- #M20004 GFP-Tag (7G9) Mouse mAb
- #M20007 GST-Tag (12G8) Mouse mAb
- #M20008 DYDDDDDK-Tag (3B9) Mouse mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody)