

Anti-6X His tag Antibody [A5D1-R]

HA601079



Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Species independent
Applications:	WB, IP
Clone number:	A5D1-R

Description: His-Tag Antibody is a high quality monoclonal His-Tag antibody (also designated 6X His-Tag antibody or His-Probe antibody) suitable for the detection of the His-Tag protein. His-Tag Antibody is available as both the non-conjugated anti-His-Tag antibody form, as well as multiple conjugated forms of anti-His-Tag antibody, including agarose, HRP, PE, FITC and multiple Alexa Fluor® conjugates. 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.

Immunogen: Synthetic peptide HHHHHHGC GHHHHHH.

Positive control: C-terminal His-tag fusion protein lysates, Mid His-tag fusion protein lysates.

Recommended Dilutions:

WB	1:5,000-1:50,000 (C-His)
WB	1:1,000-1:5,000 (Mid-His)
IP	Use at an assay dependent concentration (C-His)

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

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

Technical:0086-571-89986345

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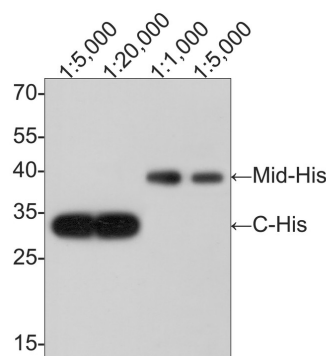
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Images

Fig1: Western blot analysis of 6X His tag on different lysates with Mouse anti-6X His tag antibody (HA601079) at different dilutions.

Lane 1/2: C-terminal His-tag fusion protein lysates

Lane 3/4: Mid His-tag fusion protein lysates



Lysates/proteins at 50 ng/Lane.

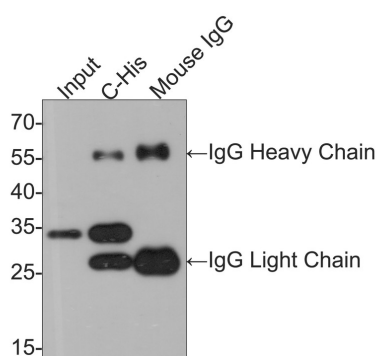
Observed band size: 30/40 kDa

Exposure time: 30 seconds;

12% 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 (HA601079) at different dilutions was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

Fig2: His-tag was immunoprecipitated in 2µg C-terminal His-tag fusion protein lysate with HA601079. Western blot was performed from the immunoprecipitate using HA601079 at 1/2,000 dilution. Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:150,000 dilution was used for 60 mins at room temperature.



Lane 1: C-terminal His-tag fusion protein lysate (input).

Lane 2: HA601079 IP in C-terminal His-tag fusion protein lysate.

Lane 3: Mouse IgG instead of HA601079 IP in C-terminal His-tag fusion protein lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

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

Background References

- Hengen, Paul N (1995). Purification of His-Tag fusion proteins from Escherichia coli. Trends in Biochemical Sciences. 20 (7): 285-6.
- Hochuli, E.; Döbeli, H.; Schacher, A. (January 1987). New metal chelate adsorbent selective for proteins and peptides containing neighbouring histidine residues. Journal of Chromatography A. 411: 177-184.

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