

Datasheet for 200-306-382**6X His Tag Antibody Biotin Conjugated****Overview**

Description:	Anti-6X His Epitope Tag (MOUSE) Monoclonal Antibody Biotin Conjugated - 200-306-382
Item No.:	200-306-382
Size:	100 µg
Applications:	ELISA, WB
Reactivity:	His-Tag
Host Species:	Mouse

Product Details**Background:**

Epitope tags are short peptide sequences that are easily recognized by tag-specific antibodies. Due to their small size, epitope tags do not affect the tagged protein's biochemical properties. Most often, sequences encoding the epitope tag are included with target DNA at the time of cloning to produce fusion proteins containing the epitope tag sequence. This allows anti-epitope tag antibodies to serve as universal detection reagents for any tag-containing protein produced by recombinant means. This means that anti-epitope tag antibodies are a useful alternative to generating specific antibodies to identify, immunoprecipitate or immunoaffinity purify a recombinant protein. The anti-epitope tag antibody is usually functional in a variety of antibody-dependent experimental procedures. Expression vectors producing epitope tag fusion proteins are available for a variety of host expression systems including bacteria, yeast, insect and mammalian cells. Rockland Immunochemicals produces anti-epitope tag antibodies against many common epitope tags including Myc, GST, GFP, 6X His, MBP, FLAG and HA. Rockland Immunochemicals also produces antibodies to other tags including FITC, Rhodamine (TRITC), DNP and biotin.

Synonyms:	mouse anti-6X His Tag biotin conjugated Antibody, biotin conjugated mouse anti-6X His Tag Antibody, anti-HIS, HIS Antibody, 6X His Tag Antibody, HHHHHH epitope tag antibody
Host Species:	Mouse
Conjugate:	Biotin
Clonality:	Monoclonal
Clone ID:	33D10.D2.G8
Format:	IgG1
F/P Ratio:	10-20

Target Details

Reactivity:	His-Tag
Immunogen Type:	Conjugated Peptide
Immunogen:	This antibody was produced in mice by repeated immunizations with 6X His epitope tag peptide H-H-H-H-H-H conjugated to KLH using maleimide.
Purity/Specificity:	Anti-6X HIS Epitope Tag was purified from concentrated tissue culture supernate by Protein A chromatography and is directed against the 6X His motif and is useful in determining its presence in various assays. This monoclonal anti-6X His tag antibody detects over-expressed proteins containing the 6X His epitope tag. To date, this antibody has reacted with all His tagged proteins so far tested. In western blotting of bacterial extracts, the antibody does not cross-react with endogenous proteins. The antibody recognizes the His-tag (His-His-His-His-His) fused to either the amino- or carboxy-termini of targeted proteins in transfected or transformed cells.

Application Details

Tested Applications:	ELISA, WB
Application Note:	Anti-6X His is optimally suited for monitoring expression of His-tagged fusion proteins. As such, anti-6X His/6X His can be used to identify fusion proteins containing the 6X His epitope. The antibody recognizes the His tag fused either to the amino- or carboxy- termini of targeted proteins. This antibody has been tested by ELISA and western blotting against both the immunizing peptide and His-containing recombinant proteins. Although not tested, this antibody is likely functional for immunoprecipitation and immunocytochemistry.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:50,000 - 1:175,000
IHC:	1:1,000 - 1:5,000
WB:	1:2,000 - 1:10,000

Formulation

Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

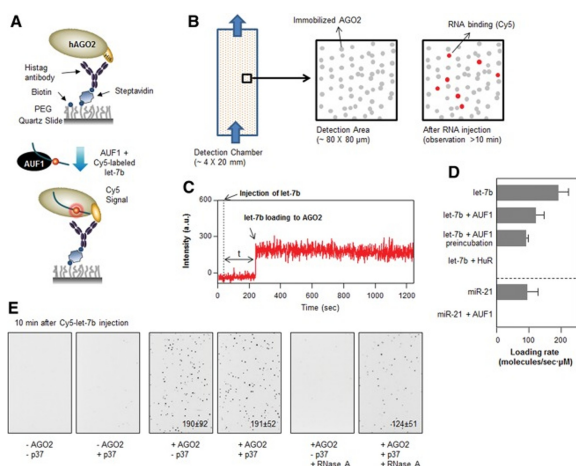
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	100 μ L
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping Condition:	Ambient
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Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
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Expiration:	Expiration date is one (1) year from date of receipt.
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Images

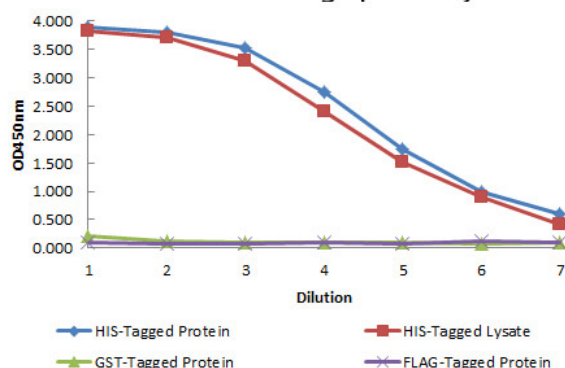


Immunocytochemistry

AUF1 promotes loading of let-7b on AGO2. (A) Schematic of recombinant AGO2 immobilization on PEG-coated slides via streptavidin and anti-His antibody (p/n 200-306-382). (B) Following the loading of Cy5-labeled let-7b RNA, the presence of label on each human AGO2 (hAGO2) molecule was assessed by measuring fluorescence intensity. (C) Representative traces of fluorescence intensity of Cy5-let-7b over time; Cy5-let-7b was injected into detection chambers with immobilized hAGO2 at 40 sec (dashed line). (D, top) Loading rate of Cy5-let-7b onto immobilized hAGO2 (binding life time >200 sec) after injecting 100 pM Cy5-let-7b alone, 100 nM AUF1 p37, 100 nM HuR along with Cy5-let-7b, or 100 nM AUF1 preincubated for 5 min with Cy5-let-7b before injection. (Bottom) Loading rate of Cy5-miR-21 onto immobilized hAGO2 (binding lifetime >200 sec) after injecting 100 pM Cy5-miR-21 alone or 100 nM AUF1 p37 along with Cy5-miR-21. (E) Cy5-let-7b spots on slides with or without immobilized AGO2, AUF1 p37, and RNase A treatment 10 min after injection. Data in C and E are representative of three independent experiments; data in D represent the means $\pm$ SD of three independent experiments. Fig 3.

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Anti-6xHIS Tag Specificity

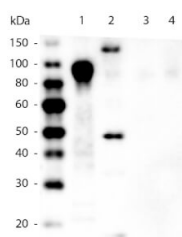


ELISA

ELISA of Mouse anti-6xHIS Tag Antibody. Antigen: HIS-tagged purified protein and E. coli cell lysates expressing HIS-Tagged construct, GST- and RON-tagged purified proteins. Coating amount: 0.15ug per well. Primary antibody: 6xHIS Tag antibody at 100ug/mL. Dilution series: 2-fold. Mid-point concentration: 200ng/mL. Secondary antibody: Peroxidase mouse secondary antibody at 1:10,000. Substrate: TMB (p/n TMBE-1000).

Western Blot

Western Blot of Mouse anti-6xHIS Tag Antibody. Lane 1: 100ng Purified histidine-tagged recombinant protein. Lane 2: 200ng E. coli cell lysate containing histidine-tagged expression construct. Lane 3: 100ng Purified GST-tagged recombinant protein. Lane 4: 100ng Purified FLAG-tagged recombinant protein. Primary antibody: Mouse anti-6xHIS Tag antibody at 1:5,000 overnight at 4°C. Secondary antibody: Peroxidase mouse secondary antibody at 1:20,000 for 30 min at RT. Block: 5% BLOTTO for 1 hr at RT.



References

- Ciferri et al. Antigenic Characterization of the HCMV gH/gL/gO and Pentamer Cell Entry Complexes Reveals Binding Sites for Potently Neutralizing Human Antibodies. *PLOS Pathogens* (2015)
- Yoon, JH et al. AUF1 promotes let-7b loading on Argonaute 2. *Genes & Development* (2015)

Disclaimer

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