Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 200-303-382

6X His Tag Antibody HRP Conjugated

Overview

Description:	Anti-6X His Epitope Tag (MOUSE) Monoclonal Antibody Peroxidase Conjugated - 200-303-382
Item No.:	200-303-382
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 HRP conjugated Antibody, peroxidase conjugated mouse anti-6X His Tag Antibody, anti-HIS, HIS Antibody, 6X His Tag Antibody, HHHHHH epitope tag antibody
Host Species:	Mouse
Conjugate:	Peroxidase (HRP)
Clonality:	Monoclonal
Clone ID:	33D10.D2.G8
Format:	IgG1

Target Details

Reactivity:	His-Tag
Immunogen Type:	Conjugated Peptide
Immunogen:	This 6X his 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:	6X His antibody is directed against the 6X His motif and was purified from concentrated tissue culture supernate by Protein A chromatography. This monoclonal anti-6X His tag antibody detects over-expressed proteins containing the 6X His epitope tag and is useful in determining its presence in various assays. 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-His) fused to either the amino- or carboxy-termini of targeted proteins in transfected or transformed cells.
Relevant Links:	• 200-303-382 SDS

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:	1:10,000
IHC:	1:500 - 1:2,500
WB:	1:1,000 - 1:5,000

Formulation

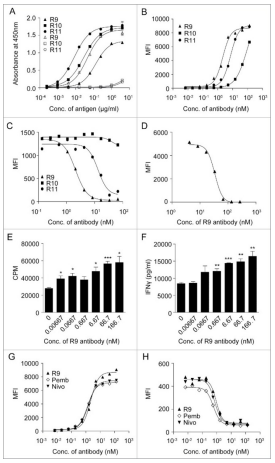
Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Gentamicin Sulfate. Do NOT add Sodium Azide!

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

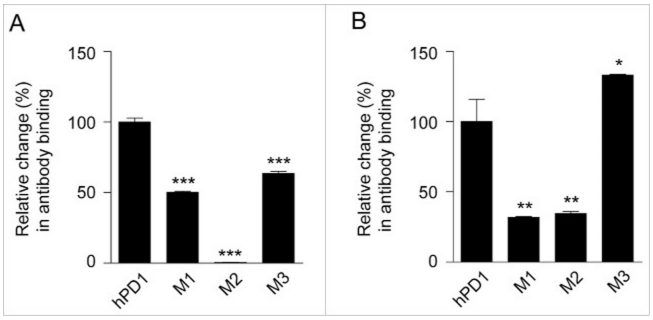
Shipping & Handling

Shipping Condition:	Ambient
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.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

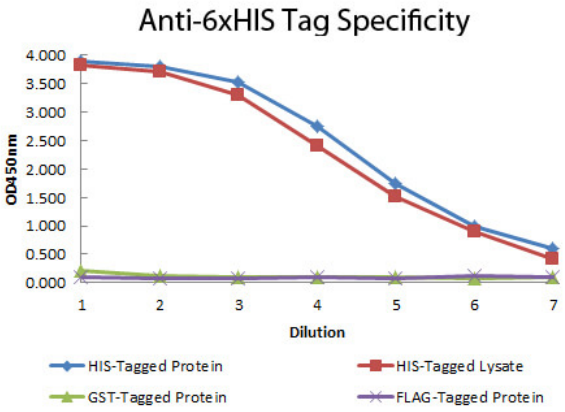


ELISA
Characterization of antibodies. (A) Cross-reactivity of anti-PD-1 antibodies to human/mouse PD-1. Each antibody was coated on 96-well plate overnight and incubated with hPD-1-His protein (filled shapes) and mPD-1-His protein (open shapes), and then HRP-conjugated anti-His antibody was added for detection. (B-D) Binding titration of the antibodies to hPD-1 expressed on CHO-S cells (B), competition of anti-PD-1 antibodies with hPD-L1 to hPD-1 expressed on CHO-S cells (C) and competition of anti-PD-1 antibody R9 with mPD-L1 to mPD-1 expressed on 293F cells were detected using FACS instrument as described in Materials and Methods. (E-F) Anti-PD-1 antibody R9 can enhance CD4+ T cell proliferation (E) and IFN-γ production (F) in a dose-dependent manner in mixed lymphocyte reaction assay as described in Materials and Methods. (unpaired, 2-tailed t test; * p< 0.05, ** p< 0.01, *** p< 0.001). (G-H) Comparison of R9, pembrolizumab (Pemb) and nivolumab (Nivo) in hPD-1 binding (G) and hPD-L1 competition (H) using FACS method as described in Materials and Methods.
Fig 1.
PMID: 28300475



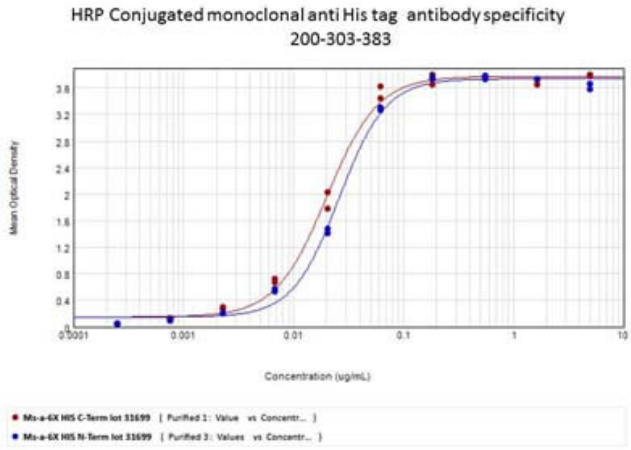
ELISA

The influences of some epitope hot spots to the capability of antibody cross-reactivity. The hot spots located on hPD-1 were changed to the corresponding mPD-1 amino acids and the binding activity of (A) R11 and (B) R9 were tested by ELISA. M1: V64M;
M2: 83PEDRSQPGQDC93 to 83CNGLSQPVQDA93; M3: A129H, Q133K, K135E. 2 µg/ml of each antibody was coated at 96-well plate overnight and incubated with (A) 10 ng/ml and (B) 150 ng/ml mutants, then HRP-anti-His antibody were added for detection. The relative binding changes of the PD-1 mutants were normalized to the original binding of antibody and hPD-1 (unpaired, 2-tailed t test; * p< 0.05, ** p< 0.01, *** p< 0.001).
Fig 6. PMID: 28300475



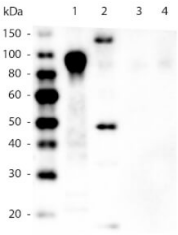
ELISA

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).



ELISA

ELISA of Mouse anti-6xHIS Tag Antibody. Antigen: 6X HIS-tagged conjugated BSA at the N or C terminus of the 6XHis. Coating amount: 0.15ug per well. Primary antibody (direct detection): HRP conjugated 6xHIS Tag antibody diluted from stock concentration at 100ug/mL. 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

- Cramer P et al. A viral glycoprotein targets IgG+ memory B cells to mediate humoral immune evasion. *EMBO Mol Med.* (2026)
- Trebosc V et al. Targeting virulence regulation to disarm Acinetobacter baumannii pathogenesis. *Virulence.* (2022)
- Lee, CH et al. An engineered human Fc domain that behaves like a pH-toggle switch for ultra-long circulation persistence. *Nature Communications* (2019)
- Li et al. Epitope mapping reveals the binding mechanism of a functional antibody cross-reactive to both human and murine programmed death 1. *mAbs* (2017)

Disclaimer