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Datasheet for 200-304-382

6X His Tag CY3 Conjugated Antibody

Overview

Description:	Anti-6X His Epitope Tag (MOUSE) Monoclonal CY3 Conjugated Antibody - 200-304-382
Item No.:	200-304-382
Size:	50 µg
Applications:	Dot Blot, ELISA, Microarray
Reactivity:	His-Tag
Host Species:	Mouse

Product Details

Background:	6X His Tag CY3 conjugated Antibody as well as other 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 CY3 conjugated Antibody, CY3 conjugated mouse anti-6X His Tag Antibody, anti-HIS, HIS Antibody, 6X His Tag Antibody, HHHHHH epitope tag antibody, CY3, Cyanine 3
Host Species:	Mouse
Conjugate:	Cy3™
Clonality:	Monoclonal
Clone ID:	33D10.D2.G8
Format:	IgG1

F/P Ratio:	8.3

Target Details

Reactivity:	His-Tag
Immunogen Type:	Conjugated Peptide
Immunogen:	HIS Tag 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 Epitope Tag CY3 conjugated antibody was purified from concentrated tissue culture supernate by Protein A chromatography 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-His) fused to either the amino- or carboxy-termini of targeted proteins in transfected or transformed cells.

Application Details

Tested Applications:	Dot Blot
Suggested Applications:	ELISA, Microarray (Based on references)
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 that contain 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 dot blot, ELISA, and western blotting against both the immunizing peptide and His-containing recombinant proteins. CY3s are designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	User Optimized
FC:	1:500 - 1:2,500
FLISA:	1:10,000 - 1:50,000
IF:	1:1,000 - 1:5,000
WB:	User Optimized

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:	50µL
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

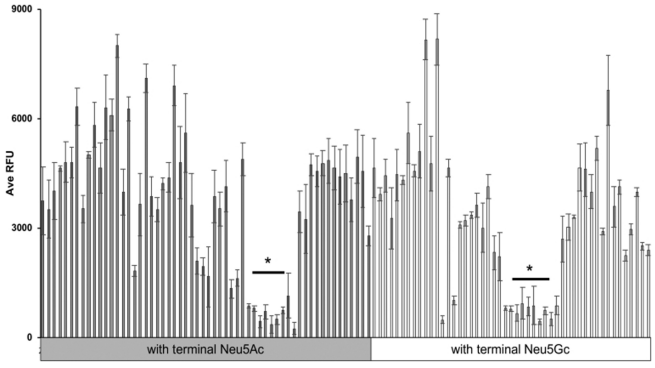
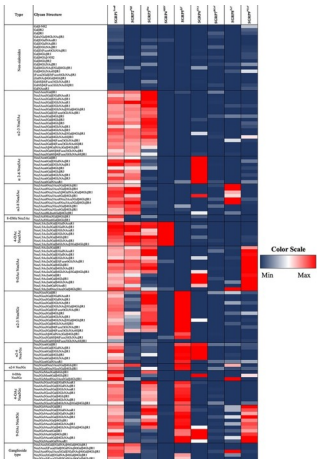


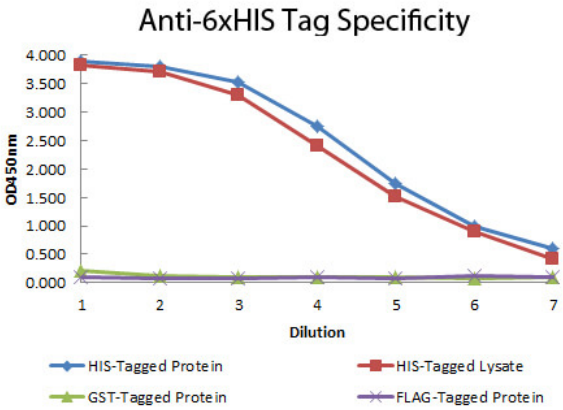
Figure
YpeB exhibits strong binding to all Neu5Ac- and Neu5Gc-terminated glycans (except 4-O-acetylated, shown by asterisk ⍰). The right-side bar shows Neu5Gc-terminated glycans, and the left-side bar shows Neu5Ac-terminated glycans. YpeB was tested on microarray in 30 µg/ml concentration. Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid. In order to determine the glycan-binding patterns of YpeB, we used a customized sialoglycan array (28) to compare the ability of His6-tagged-YpeB and fluorescently labeled anti-His secondary antibody (p/n 200-304-382) to bind pairs of sialylated glycans terminating in either Neu5Ac (prominently expressed in human cells) or Neu5Gc (prominently expressed in cells of most other mammals).
Fig 2. PMID: 35398357



Figure

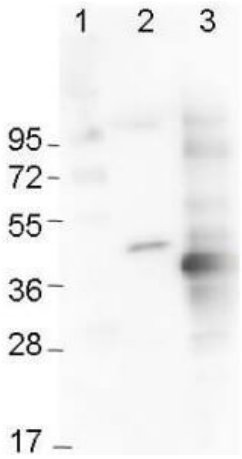
Sialoglycan microarray binding studies of proposed SGRPs. Heatmap analysis of SGRPs (see Table I for details of nomenclature) binding to mammalian (sialo) glycans; SGRP1YenB (30 µg/ml), SGRP2PltB (30 µg/ml), SGRP3Hsa (30 µg/ml), SGRP4MHV (30 µg/ml), SGRP5IgY (20 µg/ml), SGRP6SNA (20 µg/ml), SGRP7BCoV (60 µg/ml), SGRP8TeT (30 µg/ml), and SGRP9PToV (30 µg/ml) in microarray experiments. SGRPs binding efficiencies are displayed in red for highest binding (saturated or 100%), blue for minimum or no binding (0%), and intermediate binding represented by colors ranging between blue and red. Ranks; red (100%, or maximum), blue (0%, or minimum). Markedly reduced or absent binding was seen with the no-binding control probes studied simultaneously (see Fig. S4, see online supplementary material for a color version of this figure). The slides were treated with Cy3-conjugated anti-His (p/n 200-304-382) secondary antibody.

Fig 1. PMID: 35926090

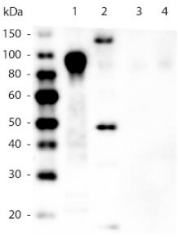


ELISA

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 using Rockland Immunochemicals' Mouse Anti-6x-His Epitope Tag Monoclonal Antibody showing detection of the 6xHis sequence on N-terminally-tagged (lane 2) and C-terminally-tagged recombinant proteins (lane 3). In lane 1 are molecular weight markers.



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

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