

Datasheet for 200-356-382

6X His Tag ATTO 647N Conjugated Antibody**Overview**

Description:	Anti-6X His Epitope Tag (MOUSE) Monoclonal ATTO 647N Conjugated Antibody - 200-356-382
Item No.:	200-356-382
Size:	50 µg
Applications:	Dot Blot, FC, IF
Reactivity:	His-Tag
Host Species:	Mouse

Product Details

Background:	6X His Tag ATTO 647N 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 ATTO 647N conjugated Antibody, ATTO 647N conjugated mouse anti-6X His Tag Antibody, anti-HIS, HIS Antibody, 6X His Tag Antibody, HHHHHH epitope tag antibody, AT647N, ATTO 647N, ATTO-TEC 647N
Host Species:	Mouse
Conjugate:	ATTO 647N
Clonality:	Monoclonal
Clone ID:	33D10.D2.G8
Format:	IgG1

F/P Ratio: 1.0

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 ATTO 647N conjugated antibody 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:	FC, IF (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 ELISA, dot blot, and western blotting against both the immunizing peptide and His-containing recombinant proteins. ATTOs are designed for STED microscopy, FRET, 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.
FLISA:	>1:20,000
IF:	>1:5,000
WB:	>1:10,000

Formulation

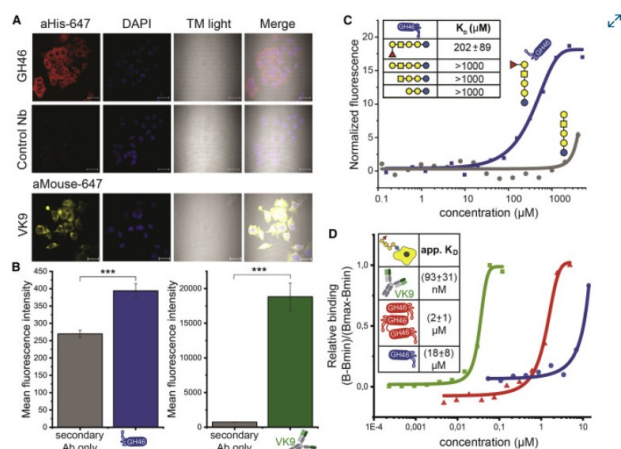
Physical State:	Lyophilized
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Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	50 μ L
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



Flow Cytometry

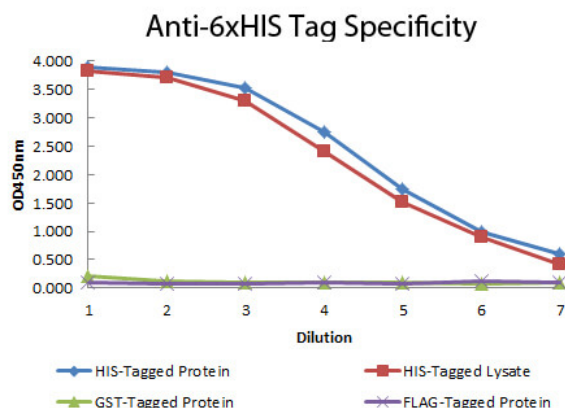
(A) Immunostaining of MCF7 cells with GH46 (red), an unrelated control Nb (red), or VK9 (yellow), and DAPI (blue) (scale bar, 5 μ m). Same pseudocolor reflects same acquisition and display settings.

(B) Flow cytometry binding assay with MCF7 cells. Left: staining of MCF7 cells with anti-6xHis-Atto647N Ab in the absence (gray) or presence (blue) of GH46. Right: staining of MCF7 cells with anti-mouse-IgG-635 Ab in the absence (gray) or presence (green) of VK9. The y axis is MFI. Values represent mean $\pm$ SEM (n = 7). Differences were tested for significance via t test. $p < 0.001$.

(C) Measured in-solution KD of GH46 and synthetic glycans (table) and comparative affinity curves for GB5 (gray) and Globo-H (blue). Values represent mean $\pm$ SEM (n $\geq$ 3).

(D) Measured apparent KD on MCF7 cells (table) and representative affinity curves for GH46 (blue), GH463 (red), and VK9 (green). Values represent mean $\pm$ SEM (n = 3).

Fig 3. (See also Figures S3 and S4.) PMID: 35705094

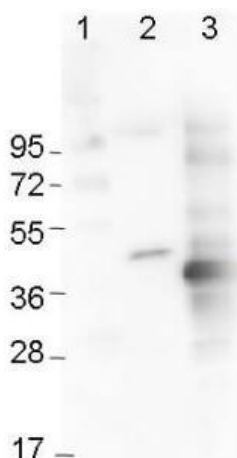


ELISA

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).

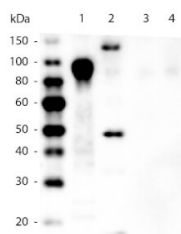
Western Blot

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

- Khilji SK et al. Generation of glycan-specific nanobodies. *Cell Chem Biol.* (2022)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.