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Datasheet for 200-343-382

# 6X His Tag Antibody DyLight™ 649

## Overview

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|---|---|
| **Description:** | Anti-6X His Epitope Tag (MOUSE) Monoclonal Antibody DyLight™ 649 Conjugated - 200-343-382 |
| **Item No.:** | 200-343-382 |
| **Size:** | 100 µg |
| **Applications:** | Dot Blot, ELISA, WB, Biochemical Assay, Microarray |
| **Reactivity:** | His-Tag |
| **Host Species:** | Mouse |

## Product Details

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| **Background:** | The emission spectra for this DyLight™ conjugate match the principle output wavelengths of most common fluorescence instrumentation. |
| **Synonyms:** | mouse anti-6X His Tag DyLight™ 649 conjugated Antibody, DyLight™ 649 conjugated mouse anti-6X His Tag Antibody, anti-HIS, HIS Antibody, 6X His Tag Antibody, HHHHHH epitope tag antibody |
| **Host Species:** | Mouse |
| **Conjugate:** | DyLight™ 649 |
| **Clonality:** | Monoclonal |
| **Clone ID:** | 33D10.D2.G8 |
| **Format:** | IgG1 |
| **F/P Ratio:** | 2.5 |

## Target Details

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| **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. |

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| **Purity/Specificity:** | This protein-A purified 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

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| **Tested Applications:** | Dot Blot, ELISA, WB |
| **Suggested Applications:** | Biochemical Assay, Microarray (Based on references) |
| **Application Note:** | Anti-6X HIS tagged Antibody conjugated to DyLight™649 has been tested by ELISA, dot blot, and western blot. This product is designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA), fluorescent polarization, FRET and fluorescent western blotting. Anti-6X HIS Antibody 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:** | >1:10,000-1:25,000 |

## Formulation

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|---|---|
| **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 µL |
| **Reconstitution Buffer:** | Restore with deionized water (or equivalent) |

## Shipping & Handling

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| **Shipping Condition:** | Ambient |

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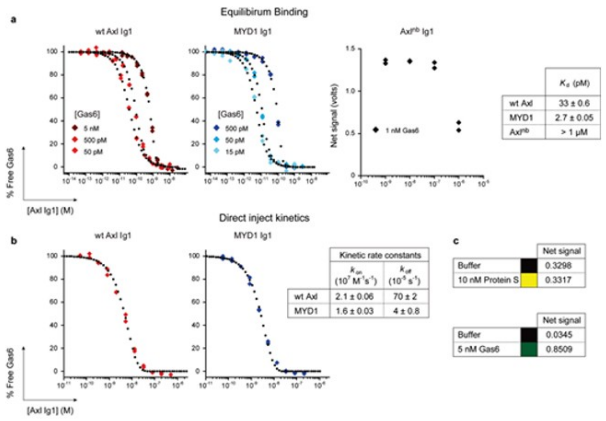
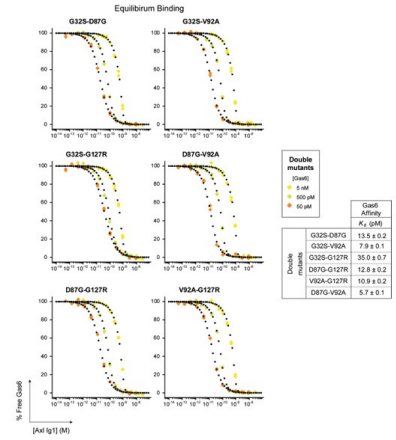
| **Storage Condition:** | Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4°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

## Images




**Figure**

KinExA measurements of Axl Ig1 variants. (a) N-curve analysis of Gas6 binding titrations for wild-type Axl Ig1, MYD1 Ig1, and Axlnb Ig1. N-curve analysis of Axlnb could not be performed as complete binding to 1 nM of Gas6 was not attained even at µM concentrations of Axlnb. This corresponds to a Kd > 1µM which is out of the measurable range of this assay. (b) KinExA direct inject kinetic data for wild-type Axl Ig1 and MYD1 Ig1. (c) KinExA signal tests of Protein S and Gas6 binding to MYD1 Fc coated beads. MYD1 Fc does not bind Protein S as no signal was detected after 700 µl of a 10 nM Protein S solution was passed over beads (left). In comparison, a robust signal was obtained after 100 µl of a 5 nM solution of Gas6 was flowed over the same beads. Curve fits are shown as dotted black lines and nominal concentrations of Gas6 are indicated.
After the appropriate incubation time, reactions were flowed over MYD1 Fc coated beads and captured free Gas6 was probed using an anti-6xHIS Dylight 649 antibody (p/n 200-343-382).
To test binding of Protein S to MYD1, 700 µl of a 10 nM solution was flowed over the capture beads. Binding of Protein S to the beads was probed using an anti-c-Myc Dylight 649 antibody (p/n 600-443-381).
Supplementary Figure 2. PMID: 25242553

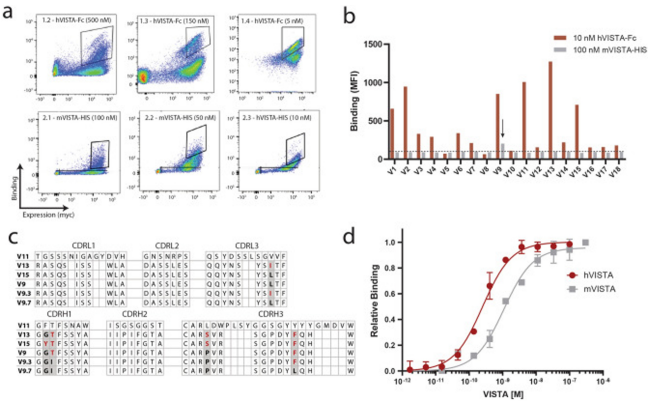



**Figure**

KinExA measurements of MYD1 Ig1 double point mutants. Ncurve analysis was performed using binding titrations to 5 nM, 500 pM, and 50 pM of Gas6 for each double mutant of MYD1 to determine the binding affinity values shown in Fig. 1e. Curve fits are shown as dotted black lines and nominal concentrations of Gas6 are indicated.

After the appropriate incubation time, reactions were flowed over MYD1 Fc coated beads and captured free Gas6 was probed using an anti-6xHIS Dylight 649 antibody (p/n 200-343-382).

Supplementary Figure 4. PMID: 25242553.




**Flow Cytometry**

Engineering a cross-reactive anti-VISTA antibody. (a) Library screening progression used to isolate scFv variants that bound human VISTA (Round 1) and mouse VISTA (Round 2). Flow cytometry gates used for screening are shown on dot plots of individual sorts. X-axis depicts expression of scFv on the yeast cell surface, y-axis depicts VISTA binding, measured by antibodies against c-myc and VISTA, respectively. (b) Binding intensity to human VISTA-Fc (red) and mouse VISTA-His (gray) of individual scFv clones isolated after Round 1 of screening. The V9 clone displayed above background binding signal to mouse VISTA-His. (c) Sequence comparison of scFv clones after the first round (V9, V11, V13, V15) and after the second round (V9.3 and V9.7) of sorting. Amino acid differences from final V9.7 clone are highlighted in red. (d) Full kinetic binding curves of SG7 (antibody form of top clone V9.7) against human VISTA-His and mouse VISTA-His monomers, as measured by KinExA. Mean ± standard deviation of duplicate measurements are shown for D. Fig 1.

PMID: 32938950

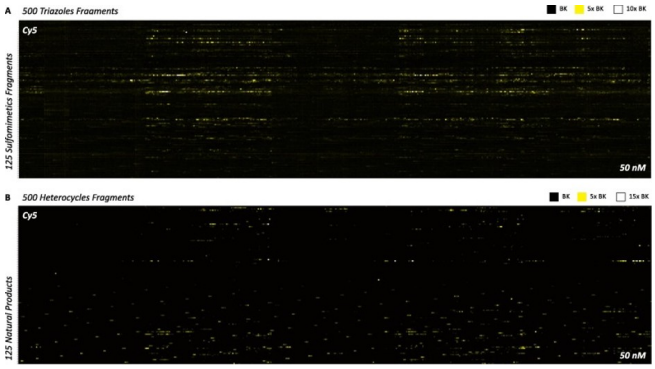
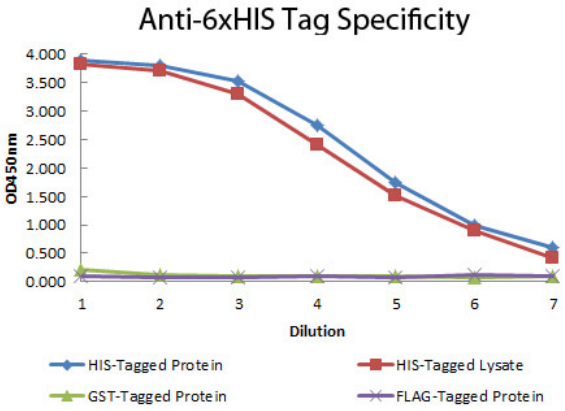


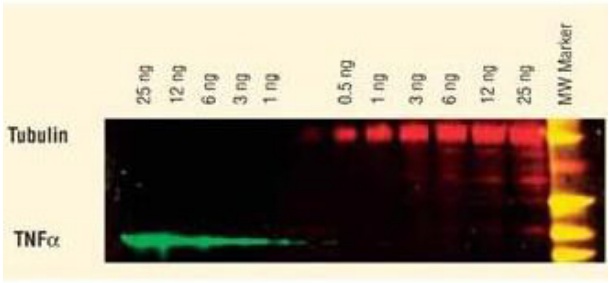

**Figure**
Bcl-XL microarray-based screening by PNA/DNA hybridization of fragment pairs combinations. Two selected examples of heat map fluorescence intensities (Cy5 channel) for the screen of 50 nM Bcl-XL with PNA encoded libraries of fragments. A) sulfomimetics fragments and triazole fragments , B) natural products and heterocycles fragments, hybridized into DNA microarrays forming combinations of non-covalently attached fragments pairs. BK = background. The heat maps have been normalized over the corresponding background for comparison purposes. The presence of the protein on the microarray features was detected by immunostaining the slide. Anti-6XHis antibody DyLight649 conjugated (p/n 200-343-382) diluted 1/5000 in PBS-T, 0.5% BSA. Fig. 2. PMID: 34216984



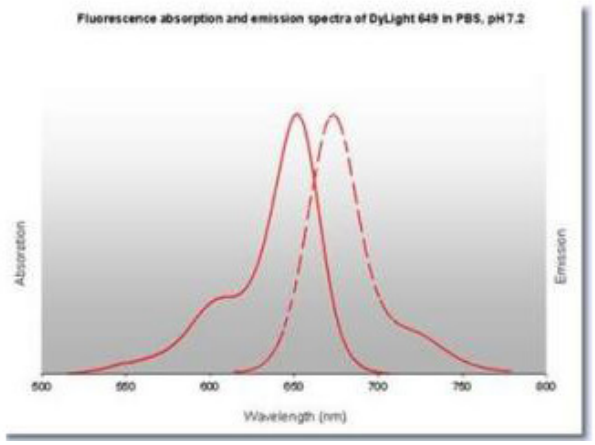

**ELISA**
ELISA of Mouse anti-6xHIS Tag Antibody. Antigen: HIS-tagged purified protein and E. coli cell lysates expressing HIS-Tagged construct, GST- and RON-tagged purified proteins. Coating amount: 0.15ug per well. Primary antibody: 6xHIS Tag antibody at 100ug/mL. Dilution series: 2-fold. Mid-point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

**Diagram**
Properties of DyLight™ Conjugates.

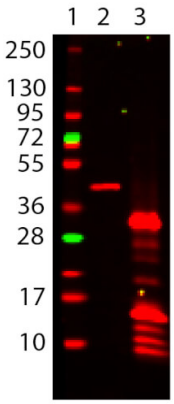
| Emission | Color | DyLight™ Dye | Ex/Em (nm) | ε ($M^{-1}$ $cm^{-1}$) | Similar Dyes |
|---|---|---|---|---|---|
| **Blue** | | **405** | **400/420** | **30,000** | **Alexa™ 405, Cascade Blue** |
| Green | | 488 | 493/518 | 70,000 | Alexa™ 488, Cy2®, FITC |
| Yellow | | 549 | 550/568 | 150,000 | Alexa™ 546, Alexa 555, Cy3®,TRITC |
| Red | | 649 | 646/674 | 250,000 | Alexa™ 647, Cy5® |
| Near Infrared | | 680 | 682/715 | 140,000 | Alexa™ 680, Cy5.5®, IRDye™ 700 |
| Infrared | | 800 | 770/794 | 270,000 | IRDye™ 800 |



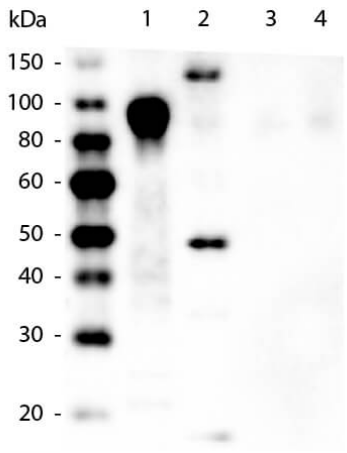

**Western Blot**
DyLight™ dyes can be used for two-color Western Blot detection with low background and high signal. Anti-tubulin was detected using a DyLight™ 549 conjugate. Anti-TNFα was detected using a DyLight™ 649 conjugate. The image was captured using the Typhoon™ 9410 Imaging System.




**Diagram**




**Western Blot**
Western Blot of Mouse Anti-6X HIS EPITOPE TAG Antibody DyLight™ 649 Conjugated. Lane 1: Molecular Weight Ladder. Lane 2: Recombinant 6xHIS-SUMO-GFP. Lane 3: Nag1. Load: 100ng Primary antibody: 6X HIS EPITOPE TAG DyLight™ 649 Conjugated at 1:1000 30 min RT. Block: 1% BSA-TTBS 30 min RT. Observed size: Lane 2: 45 kDa, Lane 3: 16 and 32 kDa. Other band(s): Lysate breakdown.




**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


- Daguer JP et al. Dual Bcl-XL/Bcl-2 inhibitors discovered from DNA-encoded libraries using a fragment pairing strategy. *Bioorg Med Chem.* (2021)
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- Mehta N et al. An engineered antibody binds a distinct epitope and is a potent inhibitor of murine and human VISTA. *Sci Rep.* (2020)
- Kariolis et al. Inhibition of the GAS6/AXL pathway augments the efficacy of chemotherapies. *Journal of Clinical Investigation* (2017)
- Kariolis MS et al. An engineered Axl'decoy receptor'effectively silences the Gas6-Axl signaling axis. *Nat Chem Biol.* (2014)


## Disclaimer