

Certificate of Analysis**Product:** IRDye® 700DX Conjugated Affinity Purified anti-Hemagglutinin (HA) EPITOPE TAG [Rabbit]**Code:** 600-430-384**Lot #** 19542**Size:** 100 µg**Physical State:** Lyophilized**Antibody Concentration:** 1.0 mg/ml (by UV absorbance at 280 nm)**Label:** IRDye® 700DX (MW 1954)**Fluorochrome/Protein Ratio:** 2.0 moles IRDye® 700DX/mole of Rabbit IgG**Absorption Wavelength:** 689 nm**Emission Wavelength:** 700 nm**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2**Stabilizer:** 10 mg/ml BSA, IgG and Protease free**Preservative:** 0.01% (w/v) Sodium Azide

Background Information: 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 biochemical properties of the tagged protein. 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.

Application(s): Fluorescence technology is widely used to detect proteins. However, many common visible fluorophores often result in considerable background fluorescence in the visible range. Visible fluorophores are rarely used for membrane-based protein detection because of this high background. IRDye® 800 and IRDye® 700DX antibody and reagent conjugates are specifically designed for protein detection methods that use longer-wavelength, near-infrared (IR) fluorophores to visualize proteins in western blotting and other applications. Very low background fluorescence in the IR range provides for a much higher signal-to-noise ratio than visible fluorophores. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. IRDye® 800 conjugates are optimized for the Odyssey® Infrared Imaging System developed by LI-COR. IRDye® 800 conjugates are also suitable for immunofluorescence microscopy using commercially available excitation/emission filters in the 780nm/820nm range. Dual simultaneous labeling in western blots or microscopy is achieved when IRDye® 800 conjugates are used in conjunction with IRDye® 700DX or Cy5.5™ conjugates. IRDye® 800 and IRDye® 700DX conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging. Anti-HA is optimally suited for monitoring the expression of HA-tagged fusion proteins. As such, anti-HA/HA can be used to identify fusion proteins containing the HA epitope. The antibody recognizes the HA epitope tag fused to the amino-terminus of targeted proteins, as is the construction in many commonly used expression vectors. This antibody has been tested by ELISA and western blotting against both the immunizing peptide and HA-containing recombinant proteins. Affinity purification of the polyclonal antibody results in very low background levels in assays and low cross-reactivity with other cellular proteins.

Recommended Dilution(s):	LI-COR Odyssey® BLOT	1:2,500 - 1:5,000
	LI-COR In-Cell Western®	1:800 - 1:1,200
	OTHER APPLICATIONS	User Optimized

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). This product is stable for several weeks at 4° C as an undiluted liquid. 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. Dilute only prior to immediate use. Expiration date is one (1) year from date of restoration.

Purity and Specificity: This affinity purified antibody is directed against the HA motif and is useful in determining its presence in various assays. This polyclonal anti-HA tag antibody detects over-expressed proteins containing the HA epitope tag. To date, this antibody has reacted with all HA-tagged proteins tested. In western blotting of bacterial extracts, the antibody does not cross-react with endogenous proteins.

Immunogen: This antibody was purified from whole rabbit serum prepared by repeated immunizations with the 9-aa epitope tag peptide YPYDVPDYA (aa 114-122) from hemagglutinin influenza conjugated to KLH using maleimide. A residue of cysteine was added to the carboxy terminal end to facilitate coupling.

Conjugation Reference: LI-COR Biosciences, Lincoln, NE

General References: Field, J., *et al.* (1988) *Mol. Cell Biol.* **8**:2159-2165.

Related Product(s):

#600-401-381	Affinity Purified Anti-Myc TAG (Rabbit)
#600-401-382	Affinity Purified Anti-6X His TAG (Rabbit)
#600-401-383	Affinity Purified Anti-FLAG (RABBIT)
#600-401-384	Affinity Purified Anti-HEMAGGLUTININ (RABBIT)
#200-101-150	IgG Fraction of Anti-LUCIFERASE (Firefly) GOAT
#600-101-200	Affinity Purified Anti-GST (GOAT)
#600-101-215	Affinity Purified Anti-GFP (GOAT)
#200-4136	IgG Fraction of Anti-BETA GALACTOSIDASE (E.coli) (RABBIT)
#600-101-098	Affinity Purified Anti-BIOTIN (GOAT)
#600-101-096	Affinity Purified Anti-FITC (GOAT)
#200-301-246	Protein A Purified Mouse Mab Anti-RHODAMINE
#200-B01-380	Protein A Purified Hamster Mab Anti-DNP
#611-703-127	HRP Anti-Rabbit IgG [H&L] MX10 (DONKEY)
#611-132-122	IRDye® 800 Anti-Rabbit IgG [H&L] MX10 (GOAT)
#600-406-381	Biotin Conjugated Affinity Purified Anti-Myc TAG (Rabbit)
#600-406-382	Biotin Conjugated Affinity Purified Anti-6X His TAG (Rabbit)
#600-406-383	Biotin Conjugated Affinity Purified Anti-FLAG (RABBIT)
#600-406-384	Biotin Conjugated Affinity Purified Anti-HEMAGGLUTININ (RABBIT)
#200-106-150	Biotin Conjugated IgG Fraction of Anti-LUCIFERASE (Firefly) GOAT
#600-106-200	Biotin Conjugated Affinity Purified Anti-GST (GOAT)
#600-106-215	Biotin Conjugated Affinity Purified Anti-GFP (GOAT)
#200-4636	Biotin Conjugated IgG Fraction of Anti-BETA GALACTOSIDASE (E.coli) (RABBIT)
#600-106-096	Biotin Conjugated Affinity Purified Anti-FITC (GOAT)

USDA Certification: 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.

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