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### Certificate of Analysis

**Product:** IRDye™ 800CW Conjugated Affinity Purified anti-Hemagglutinin (HA) EPI TOPE TAG (Rabbit)

**Code:** 600-431-384

**Lot #** 19833

**Size:** 100 µg

**Physical State:** Lyophilized

**Antibody Concentration:** 1.0 mg/ml (by UV absorbance at 280 nm)

**Label:** IRDye™ 800CW (MW 1166.2)

**Fluorochrome/Protein Ratio:** 2.6 moles IRDye™ 800CW /mole of Rabbit IgG

**Absorption Wavelength:** 774 nm (in PBS)

**Emission Wavelength:** 800 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 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.

**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 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 Cy5.5™ conjugates. IRDye™ 800 conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging. IRDye™ 800CW conjugates are specifically designed for LI-COR's In-Cell Western assay for the Odyssey® Infrared Imaging System. IRDye™ 800CW conjugates can be used for western blotting applications, but the fluorochrome/protein ratio is not optimal for this application so detection sensitivity may be reduced. The In-Cell Western assay is a faster method for analyzing signal transduction pathways. In less time than a gel-based Western, you can quantify proteins in a 96- or 384-well microplate. In-Cell Westerns use infrared dye-labeled secondary antibodies to directly label proteins in fixed cultured cells, and quantify total fluorescence from each well. Time consuming and error-prone steps such as lysate preparation, gel loading and electrophoresis, and membrane transfer are eliminated with In-Cell Westerns. Simultaneous, two-target detection enables quantitative and accurate measurement of abundance or phosphorylation of one target by normalization against another target. Near-infrared probes yield high sensitivity for measuring small changes in protein amount or modification. Direct detection of proteins in their cellular context eliminates artifacts caused by cell lysis. The fast, microplate-based assay eliminates lysates, gels, and membranes required for conventional Western blots. The In-Cell Western assay is a moderate

throughput method, ideal for screening cell treatments or drug candidates for their effects on target proteins. Recommended plates for In-Cell Westerns are Nunc-Nalgene (p/n 167008) for 96-well format and Falcon (BD Biosciences p/n 353961) for 384-well format. Primary and secondary antibodies should be validated by Odyssey western blotting *before* performing the In-Cell Western assay to confirm specificity and performance in the infrared system. 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 expressed in many commonly used expression vectors.

|                                 |                                |                    |
|---------------------------------|--------------------------------|--------------------|
| <b>Recommended Dilution(s):</b> | <b>LI-COR Odyssey® BLOT</b>    | 1:5,000 - 1:25,000 |
|                                 | <b>LI-COR In-Cell Western®</b> | 1:800 - 1:1,200    |
|                                 | <b>OTHER APPLICATIONS</b>      | User Optimized     |

**Storage Conditions:** Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). Centrifuge product if not completely clear after standing at room temperature. This product is stable at 4° C as an undiluted liquid. 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 HA epitope tag 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 so far tested. The antibody recognizes the HA-tag (Tyr-Pro-Tyr- Asp-Val-Pro-Asp-Tyr-Ala) fused to either the amino- or carboxy- termini of targeted proteins in transfected or transformed cells.

**Immunogen:** This antibody was purified from whole rabbit serum prepared by repeated immunizations with the 9-aa epitope tag peptide YPYDVPDYA (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.

**Related Product(s):**

|                              |  |
|------------------------------|--|
| <a href="#">#600-101-098</a> | Affinity Purified Anti-BIOTIN (GOAT)       |
| <a href="#">#600-401-382</a> | Affinity Purified Anti-6X HIS TAG (Rabbit) |
| <a href="#">#600-101-200</a> | Affinity Purified Anti-GST (GOAT)          |
| <a href="#">#600-101-215</a> | Affinity Purified Anti-GFP (GOAT)          |
| <a href="#">#600-101-096</a> | Affinity Purified Anti-FITC (GOAT)         |
| <a href="#">#200-301-246</a> | Protein A Purified Mouse Mab Anti-TRITC    |
| <a href="#">#200-B01-380</a> | Protein A Purified Hamster Mab Anti-DNP    |
| <a href="#">#611-703-127</a> | HRP Anti-Rabbit IgG [H&L] MX10 (DONKEY)    |
| <a href="#">#611-132-122</a> | IRDye800 Anti-Rabbit IgG [H&L] MX10 (GOAT) |

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