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### Certificate of Analysis

**Product:** Affinity Purified anti-Hemagglutinin (HA) EPI TOPE TAG [Rabbit]

**Code:** 600-401-384

**Lot #** 21344

**Size:** 100 µg

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 0.83 mg/ml (by UV absorbance at 280 nm)

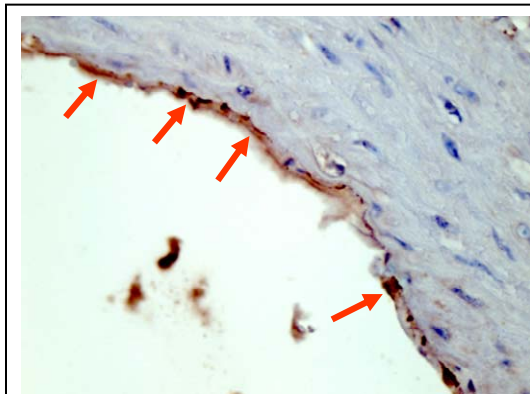
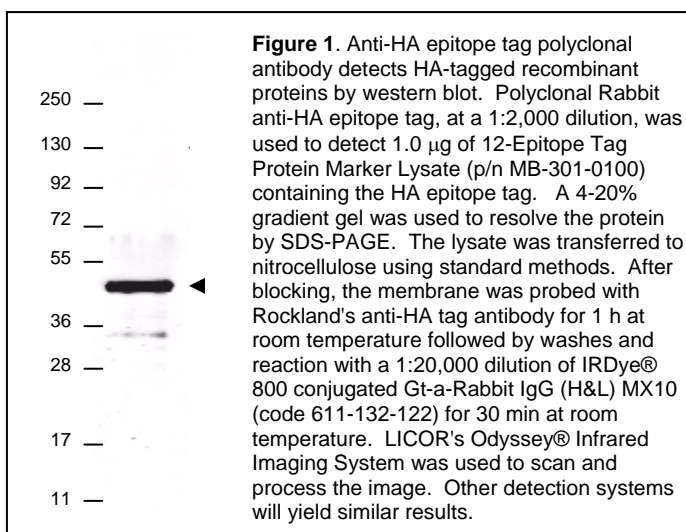
**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions:** Store vial at -20° C. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

**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 the 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.



**Figure 2.** Rockland's Affinity Purified anti- HA epitope tag polyclonal antibody detects HA tagged recombinant proteins by IHC on formalin fixed paraffin embedded tissue. Arrowheads point to expression of HA tagged proteins in endothelial cells of mouse aorta. Sections of 4 µm were prepared from representative paraffin blocks. Sections were then deparaffinized and rehydrated with xylene and alcohol. Citrate buffer antigen retrieval was performed for 30 min in a boiling jar. Anti-HA was diluted in blocking buffer at 1:2,000 and reacted at 4° C overnight followed by signal detection using horseradish peroxidase with DAB as the chromogenic substrate. Tissue was counterstained with Mayer's hematoxylin. *Personal Communication, Behzad Yeganeh, U. Manitoba, Winnipeg, Canada.*

**Application Note(s):** 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- or carboxy- termini of targeted proteins, as expressed 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. Although not tested, this antibody is likely functional for immunoprecipitation, immunocytochemistry, and other immunodetection techniques. Affinity purification of the polyclonal antibody results in very low background levels in assays and low cross-reactivity with other cellular proteins.

|                               |                             |                      |
|-------------------------------|-----------------------------|----------------------|
| <b>Recommended Dilutions:</b> | <b>ELISA</b>                | 1:20,000 - 1:150,000 |
|                               | <b>WESTERN BLOT</b>         | 1:2,000 - 1:10,000   |
|                               | <b>IMMUNOHISTOCHEMISTRY</b> | 1:500 - 1:2,000      |
|                               | <b>OTHER APPLICATIONS</b>   | User Optimized       |

**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 (114-122) from hemagglutinin influenza conjugated to KLH using maleimide. A residue of cysteine was added to the carboxy terminal end to facilitate coupling.

#### Related Product(s):

|                              |  |                              |  |
|------------------------------|--|------------------------------|--|
| <a href="#">#600-401-382</a> | Affinity Purified Anti-6X His TAG (RABBIT)       | <a href="#">#600-401-384</a> | Affinity Purified Anti-HEMAGGLUTININ (RABBIT)  |
| <a href="#">#200-4136</a>    | IgG Fraction of Anti-BETA GAL (E.coli) (RABBIT)  | <a href="#">#200-101-150</a> | IgG Fraction of Anti-LUCIFERASE (Firefly) GOAT |
| <a href="#">#600-101-098</a> | Affinity Purified Anti-BIOTIN (GOAT)             | <a href="#">#200-401-385</a> | IgG Fraction of Anti-MBP TAG (RABBIT)          |
| <a href="#">#100-401-463</a> | Anti-Chloramphenicol-Acetyl Transferase (RABBIT) | <a href="#">#600-401-381</a> | Affinity Purified Anti-Myc TAG (RABBIT)        |
| <a href="#">#200-B01-380</a> | Protein A Purified Hamster Mab Anti-DNP          | <a href="#">#200-301-246</a> | Protein A Purified Mouse Mab Anti-RHODAMINE    |
| <a href="#">#600-101-096</a> | Affinity Purified Anti-FITC (GOAT)               | <a href="#">#600-401-386</a> | Affinity Purified Anti-VSV-G TAG (RABBIT)      |
| <a href="#">#600-401-383</a> | Affinity Purified Anti-FLAG (RABBIT)             | <a href="#">#611-132-122</a> | IRDye800 Anti-RABBIT IgG [H&L] MX10 (GOAT)     |
| <a href="#">#600-101-215</a> | Affinity Purified Anti-GFP (GOAT)                | <a href="#">#611-703-127</a> | HRP Anti-RABBIT IgG [H&L] MX10 (DONKEY)        |
| <a href="#">#600-101-200</a> | Affinity Purified Anti-GST (GOAT)                |                              |  |

#### General References:

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

**Note:** 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 326, Gilbertsville, Pennsylvania, USA.