

## Product Specification Sheet

**Product:** Biotin Conjugated Affinity Purified anti-Hemagglutinin (HA) EPITOPE TAG (Rabbit)

**Code:** 600-406-384

**Lot #:** 13606

**Size:** 100 µg

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

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

**Label:** Biotinamidocaproate N-Hydroxysuccinimide Ester (BAC)

**Biotin/Protein Ratio:** 10-20 BAC molecules per Rabbit IgG molecule

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

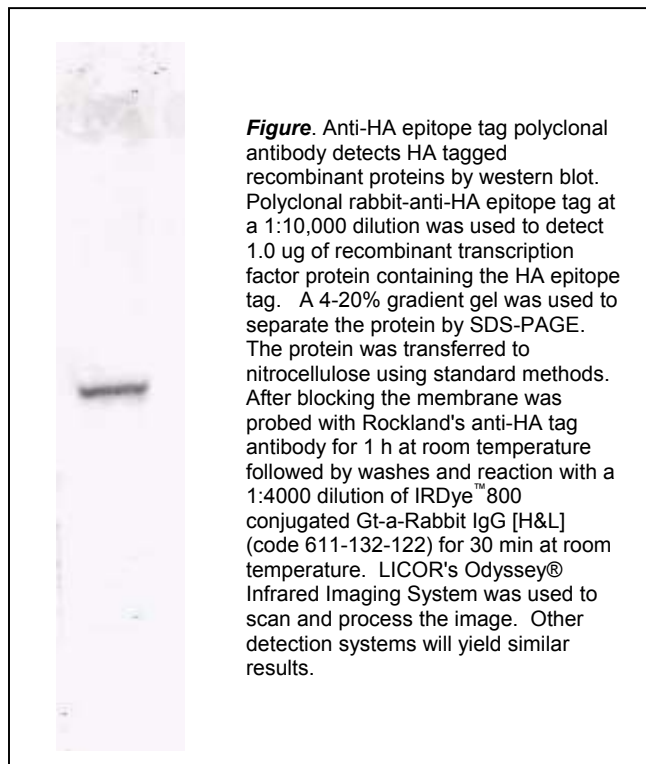
**Stabilizer:** 10 mg/ml Bovine Serum Albumin (BSA) IgG and Protease free

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

**Storage Conditions:** Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). For extended storage mix with glycerol to 50% and then 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 date is one (1) year from date of restoration.

**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 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- terminus of targeted proteins as is 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 and 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. Biotin conjugated anti-EPITOPE tag antibodies are generally suitable for immunoblotting, ELISA, immunohistochemistry, immunomicroscopy as well as other antibody based assays using streptavidin or avidin conjugates.



**Figure.** Anti-HA epitope tag polyclonal antibody detects HA tagged recombinant proteins by western blot. Polyclonal rabbit-anti-HA epitope tag at a 1:10,000 dilution was used to detect 1.0 µg of recombinant transcription factor protein containing the HA epitope tag. A 4-20% gradient gel was used to separate the protein by SDS-PAGE. The protein was transferred to nitrocellulose using standard methods. After blocking the membrane was probed with Rockland's anti-HA tag antibody for 1 h at room temperature followed by washes and reaction with a 1:4000 dilution of IRDye™ 800 conjugated Gt-a-Rabbit IgG [H&L] (code 611-132-122) for 30 min at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.

**Recommended Dilutions:** This product was assayed by immunoblot and was found to be reactive at 1:10,000. For ELISA a dilution of 1:15,000 to 1: 150,000 is suggested for detection of HA containing recombinant proteins or for screening in microtiter plates of recombinants. In general, a 1:250 dilution is recommended for immunohisto-chemistry and immunoprecipitation, but specific experimental conditions may require adjustments in dilution. Use Rockland Immunochemical's HRP Anti-RABBIT IgG (H&L) (DONKEY) MX Bv Ch Gt GP Hs Hu Ms Rt & Sh (code 611-703-127) as a secondary antibody for detection. The researcher should determine optimal titers for other applications.

**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 so far tested. In western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-biotin and anti-Rabbit Serum.

**Immunogen:** This antibody was purified from whole rabbit serum prepared by repeated immunizations with the 9-aa epitope tag peptide YPYDVPDYA (11 114-122) from hemagglutinin influenza conjugated to KLH using maleimide. A residue of cysteine was added to the carboxy terminal end to facilitate coupling.

**Conjugation Reference:** Bayer & Wilchek *Methods in Enzymology* **184**; 138-160, 1990.

#### 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	IRDye800 Anti-Rabbit IgG [H&L] MX10 (GOAT)
#600-406-381	Biotin Conjugated Affinity Purified Anti-Myc TAG (Rabbit) <b>NEW!</b>
#600-406-382	Biotin Conjugated Affinity Purified Anti-6X His TAG (Rabbit) <b>NEW!</b>
#600-406-383	Biotin Conjugated Affinity Purified Anti-FLAG (RABBIT) <b>NEW!</b>
#600-406-384	Biotin Conjugated Affinity Purified Anti-HEMAGGLUTININ (RABBIT) <b>NEW!</b>
#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)

#### General References:

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

**Conjugation Reference:** Bayer & Wilchek *Methods in Enzymology* **184**; 138-160, 1990.

**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.