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AP09230PU-N Polyclonal Antibody to HA Epitope Tag (YPYDVPDYA) - Aff - Purified

Quantity: 0.1 mg

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

Background: 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.

Host / Isotype: Rabbit / IgG

Immunogen: 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.

Format:
State: Liquid (sterile filtered) Ig fraction
Purification: Affinity Chromatography
Buffer System: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservatives: 0.01% (w/v) Sodium Azide
Stabilizers: None

Applications: 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.
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.
Recommended Dilutions
ELISA: 1/10,000-1/100,000.
Western blot: 1/2,000-1/10,000.
Immunohistochemistry: 1/500-1/2,000.

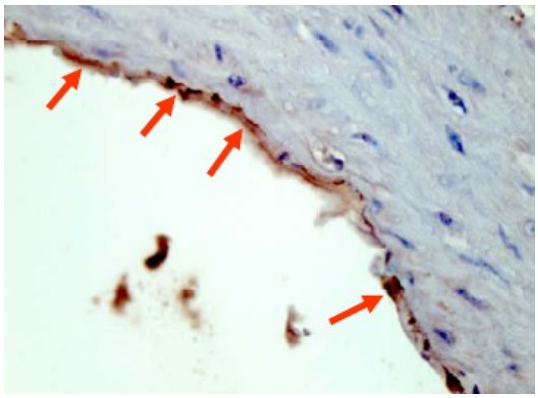
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

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

Storage: Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

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

Pictures:



Immunohistochemistry: 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.

Western blot: Anti-HA epitope tag antibody detects HA-tagged recombinant proteins. Polyclonal Rabbit anti-HA epitope tag, at a 1/2,000 dilution, was used to detect 1.0 µg of 12-Epitope Tag Protein Marker Lysate containing the HA epitope tag. A 4-20% gradient gel was used to resolve the protein by SDS-PAGE. The lysate was transferred to nitrocellulose using standard methods. After blocking, the membrane was probed with anti-HA tag antibody for 1 h at room temperature followed by washes and reaction with a 1:20,000 dilution of IRDye(R) 800 conjugated Gt-anti-Rabbit IgG (H&L) MX10 for 30 min at RT. LICOR's Odyssey(R) Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.