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R1181B Polyclonal Antibody to 6xHistidine Epitope Tag (HHHHHH) - Biotin

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 tagged proteins 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.

Host: Rabbit

Immunogen: 6X His epitope tag peptide H-H-H-H-H-H conjugated to KLH using maleimide

Format:
State: Lyophilized purified Ig fraction
Purification: Affinity Chromatography
Buffer System: 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2
Preservatives: 0.01% (w/v) Sodium Azide
Stabilizers: 10 mg/ml BSA
Label: Biotin – Biotinamidocaproate N-Hydroxysuccinimide Ester (BAC)
Molar Ratio: 10-20 BAC molecules per Rabbit IgG molecule
Reconstitution: Restore with 0.1 ml of deionized water (or equivalent).
For extended storage, mix with Glycerol to 50% final

Applications: Anti-6X His is optimally suited for monitoring expression of His-tagged fusion proteins.
As such, anti-6X His/6X His can be used to identify fusion proteins containing the 6X His epitope.
This antibody has been tested by ELISA and Western blotting against both the immunizing peptide and His-containing recombinant proteins.
Although not tested, this antibody is likely functional for Immunoprecipitation and Immunocytochemistry.
Recommended Dilutions:
ELISA: 1/10,000-1/50,000.
Western blot: 1/2,000-1/10,000.
Immunohistochemistry: 1/1,000-1/5,000.

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 6X His motif and is useful in determining its presence in various assays. This polyclonal anti-6X His-tag antibody detects over-expressed proteins containing the 6X His epitope tag. To date, this antibody has reacted with all His-tagged proteins tested.
In Western blotting of bacterial extracts, the antibody does not cross-react with endogenous proteins.
The antibody recognizes the His-tag (*His-His-His-His-His-His*) fused to either the amino- or carboxy- termini of targeted proteins in transfected or transformed cells.

Storage:

Prior to reconstitution store at 2-8°C.
Following reconstitution store the antibody 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. Bayer & Wilchek, Methods in Enzymology, 184; 138-160, 1990 (Conjugation).

Pictures:

Anti-6X His epitope tag polyclonal antibody detects His-tagged recombinant proteins by western blot. Polyclonal Rabbit-anti-6X His epitope tag at 0.5-1.0 µg/ml was used to detect 1.0 µg of recombinant protein containing the His epitope tag. A 4-20% gradient gel was used to resolve the protein by SDS-PAGE. The protein was transferred to nitrocellulose using standard methods. After blocking, the membrane was probed with the primary antibody for 1 h at room temperature followed by washes and reaction with a 1:2500 dilution of IRDye(R) 800 conjugated Gt-a-Rabbit IgG [H&L] MX10 for 30 min at room temperature. LICORs Odyssey(R) Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.