

R1180B

Polyclonal Antibody to DYKDDDDK Epitope Tag - Biotin

Alternate names:

D-tag, ECS Epitope Tag, ECS-tag, FLAG Epitope Tag, FLAG-tag

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:

This antibody was purified from whole rabbit serum prepared by repeated immunizations with the Enterokinase Cleavage Site (ECS) peptide DYKDDDDK (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) conjugated to KLH using maleimide. Residues of Glycine and cysteine were added to the carboxy terminal end to facilitate coupling.

Format:

State: Lyophilized purified 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: 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%.

Applications:

This antibody is optimally suited for monitoring the expression of FLAG™ tagged fusion proteins. As such, this antibody can be used to identify fusion proteins containing the FLAG™ epitope. The antibody recognizes the epitope tag fused to the carboxy or amino- terminus of targeted proteins. This antibody has been tested by **ELISA** and **Western blotting** against both the immunizing peptide and FLAG™ containing recombinant proteins. Although not tested, this antibody is likely functional for **Immunoprecipitation** and **Immunocytochemistry**, and other immunodetection techniques. The epitope tag peptide sequence was first derived from the 11-amino-acid leader peptide of the gene-10 product from bacteriophage T7. Now the most commonly used hydrophilic octapeptide is DYKDDDDK. This Immunochemical's polyclonal antibody to detect FLAG™ conjugated proteins binds FLAG™ containing fusion proteins with greater affinity than the widely used

monoclonal M1, M2 and M5 clones, and shows greater sensitivity in most assays. 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: 1/10,000-1/50,000.

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 antibody is directed against the FLAG™ motif and is useful in determining its presence in various assays.

This polyclonal anti-FLAG™ tag antibody detects over-expressed proteins containing the FLAG™ epitope tag. To date this antibody has reacted with all amino-terminal FLAG™ tagged proteins so far tested. In Western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins.

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. Chubet RG, Brizzard BL. Vectors for expression and secretion of FLAG epitope-tagged proteins in mammalian cells. *Biotechniques*. 1996 Jan;20(1):136-41. PubMed PMID: 8770418.
2. Sliotstra JW, Kuperus D, Plückthun A, Meloen RH. Identification of new tag sequences with differential and selective recognition properties for the anti-FLAG monoclonal antibodies M1, M2 and M5. *Mol Divers*. 1997;2(3):156-64. PubMed PMID: 9238646.
3. Robeva AS, Woodard R, Luthin DR, Taylor HE, Linden J. Double tagging recombinant A1- and A2A-adenosine receptors with hexahistidine and the FLAG epitope. Development of an efficient generic protein purification procedure. *Biochem Pharmacol*. 1996 Feb 23;51(4):545-55. PubMed PMID: 8619901.
4. Fulton JE, Thacker EL, Bacon LD, Hunt HD. Functional analysis of avian class I (BFIV) glycoproteins by epitope tagging and mutagenesis in vitro. *Eur J Immunol*. 1995 Jul;25(7):2069-76. PubMed PMID: 7621880.