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R1180 Polyclonal Antibody to DYKDDDDK Epitope Tag - Aff - Purified

Alternate names: D-tag, ECS Epitope Tag, ECS-tag, FLAG Epitope Tag, FLAG-tag

Quantity: 0.25 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 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.
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.

Host: Rabbit

Immunogen: This antibody was purified from whole rabbit serum prepared by repeated immunizations with the DYKDDDDK epitope tag peptide (Asp-Tyr-Lys-Asp-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:** Liquid (sterile filtered) purified Ig fraction
Purification: Affinity purification of this polyclonal antibody results in very low background levels in assays and low cross-reactivity with other cellular proteins.
Buffer System: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservatives: 0.01% Sodium Azide

Applications: This antibody is optimally suited for monitoring the expression of DYKDDDDK-tagged fusion proteins. As such, this antibody can be used to identify fusion proteins containing the DYKDDDDK epitope. The antibody recognizes the epitope tag fused to either the amino- or carboxy termini of targeted proteins. This antibody has been tested by ELISA and western blotting against both the immunizing peptide and DYKDDDDK containing recombinant proteins. Although not tested, this antibody is likely functional for immunoprecipitation and immunocytochemistry, and other immunodetection techniques. This polyclonal antibody to detect DYKDDDDK-conjugated proteins binds DYKDDDDK-containing fusion proteins with greater affinity than the widely used monoclonal M1, M2 and M5 clones, and shows greater sensitivity in most assays.
Recommended Dilutions:
ELISA: 1/90,000-1/250,000.

Western blot: 1/2,000-1/10,000.
Immunohistochemistry: User-optimized
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 DYKDDDDK motif and is useful in determining its presence in various assays.
This polyclonal anti-DYKDDDDK tag antibody detects over-expressed proteins containing the DYKDDDDK epitope tag. To date this antibody has reacted with all amino-terminal DYKDDDDK tagged proteins so far tested. In western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins.

Storage:

Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C or below for longer.
Avoid repeated freezing and thawing.
Dilute only prior to immediate use.
Shelf life: one year from despatch.

General Readings:

Chubet, R.G. and Brizzard, B.L. (1996) Vectors for expression and secretion of FLAG epitope-tagged proteins in mammalian cells. Biotechniques 20(1):136-141.
Slootstra, J.W. et al., (1997) Identification of new tag sequences with differential and selective recognition properties for the anti-FLAG monoclonal antibodies M1, M2 and M5. Mol Divers 2(3):156-164.
Robeva, A.S. et al., (1996) Double tagging recombinant A1- and A2A-adenosine receptors with hexahistidine and the FLAG epitope. Development of an efficient generic protein purification procedure. J Biochem Pharmacol 51:4, 545-55.
Fulton, J.E. et al., (1995) Functional analysis of avian class I (BFIV) glycoproteins by epitope tagging and mutagenesis in vitro. Eur J Immunol. 25(7): 2069-2076.

Pictures:

Western blot using the antibody at a dilution of 1:2,500 to detect 1.0 µg of recombinant protein containing the DYKDDDDK epitope tag. This antibody will detect both amino and carboxy terminal linked DYKDDDDK recombinant proteins. 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 1:10,000 dilution of IRDye(R) 800 conjugated Gt-a-Rabbit IgG (H&L) for 30 min at room temperature. LICOR's Odyssey(R) Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.