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AM08434PU-S Monoclonal Antibody to DYKDDDDK Epitope Tag - Purified

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

Quantity: 25 µl

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

Background: 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.

Host / Isotype: Mouse / IgG2a

Recommended Isotype Controls: AM03096PU-N

Clone: 29E4.G7.H11

Immunogen: Produced in Mice by repeated immunizations with a synthetic peptide corresponding to the FLAG™ epitope tag peptide DYKDDDDK (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) conjugated to KLH using maleimide.
Remarks: Residues of Glycine and Cysteine were added to the termini to facilitate coupling.

Format: **State:** Liquid (sterile filtered) purified IgG fraction.
Purification: Protein A Chromatography followed by extensive dialysis against the buffer.
Buffer System: 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2 containing 0.01% (w/v) Sodium Azide as preservative.

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 either the amino- or carboxy-termini of targeted proteins.
The epitope tag peptide sequence was first derived from the 11-amino-acid leader peptide of the gene-10 product from bacteriophage T7.
DYKDDDDK is the most commonly used hydrophilic octapeptide tag.
Recommended Dilutions:
ELISA: 1/20,000-1/100,000.
Western Blot: 1/2,000-1/20,000.

Flow Cytometry: 1/2,000-1/10,000.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

Carboxy and amino terminal linked FLAG™ tagged recombinant proteins.
This antibody is directed against the FLAG™ motif and is useful in determining its presence in various assays where the epitope tag is present at either the amino or carboxy terminus of recombinant proteins.
This monoclonal anti-FLAG tag antibody detects over-expressed proteins containing the FLAG™ epitope tag.
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 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. Slootstra 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.

Pictures:

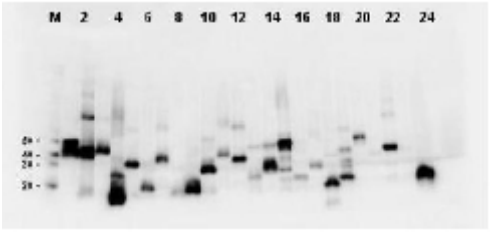

Figure 2. Monoclonal Antibody to detect FLAG conjugated proteins. Twenty-four (24) clones were randomly selected and grown up from gylcerol stocks by inoculating 0.5 mL 2xYT medium. Expression of recombinant proteins was induced by the addition of IPTG. Proteins were purified by nickel affinity chromatograaphy and eluted in 40 µL. Samples were diluted 10-fold, transferred to nitrocellulose membrane and blotted using Mab-anti-FLAG™ antibody. *Personal Communication: A. Morrison and B. Kloss, NYCOMPS, New York, NY.*

Figure 1. Affinity purified Antibody to detect FLAG(TM) conjugated proteins detects both C terminal linked and N terminal linked FLAG(TM) tagged recombinant proteins by western blot