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Datasheet for 200-301-B13

DYKDDDDK Tag (Anti-FLAG®) Antibody

Overview

Description:	Antibody for the detection of FLAG® conjugated proteins (MOUSE) Monoclonal Antibody - 200-301-B13
Item No.:	200-301-B13
Size:	500 µg
Applications:	ELISA, WB, FC, IF, IP
Reactivity:	FLAG-Tag
Host Species:	Mouse

Product Details

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. Rockland Immunochemicals produces anti-epitope tag antibodies against many common epitope tags including Myc, GST, GFP, 6X His, MBP, FLAG™ and HA. Rockland Immunochemicals also produces antibodies to other tags including FITC, Rhodamine (TRITC), DNP and biotin.
Synonyms:	mouse anti-FLAG™ tag, Enterokinase Cleavage Site (ECS), mouse anti-DYKDDDDK, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	29E4.G7
Format:	IgG2a

Target Details

Reactivity:	FLAG-Tag
Immunogen Type:	Conjugated Peptide
Immunogen:	This antibody was 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.
Purity/Specificity:	This product is an IgG fraction antibody purified from ascites by Protein A chromatography followed by extensive dialysis against the buffer stated above. The purified 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.

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	FC, IF, IP (Based on references)
Application Note:	Anti-FLAG antibody has been tested by ELISA and western blot and 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:150,000 - 1:250,000
FC:	User Optimized
IHC:	1:1,000-1:5,000
WB:	1:2,000-1:10,000

Formulation

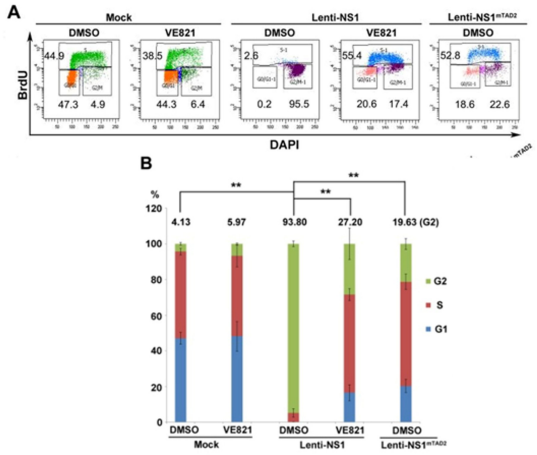
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm

Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

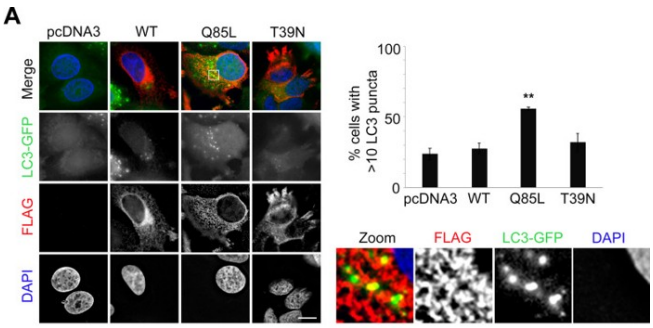
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

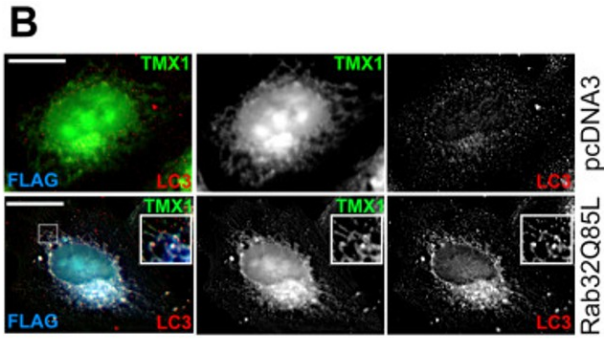
Images



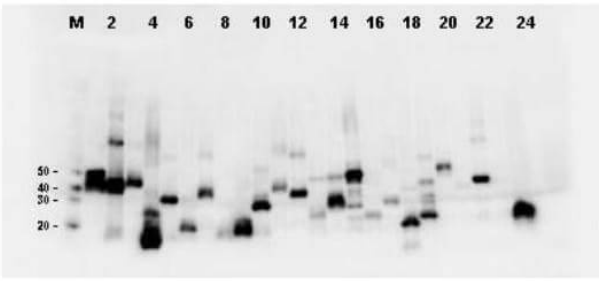
Flow Cytometry
Inhibition of ATR activation inhibits the NS1-induced G2-phase arrest of CD36+ EPCs. (A) Cell cycle analysis. CD36+ EPCs were treated with VE821 at 3 h prior to NS1-expressing lentivirus transduction or mock transduction. After 48 h, cells were collected, and the cell cycle phase of NS1-expressing cells (selected by staining with anti-Flag) was examined by flow cytometry. (B) Statistical analyses. The percentage of cells treated with DMSO or VE821 that were at G1-, S-, and G2-phase is depicted in color. The numbers shown are the percentages of the cells at G2-phase, and are statistically compared as indicated. ** P<0.01. Fig 8. PMID: 28264028



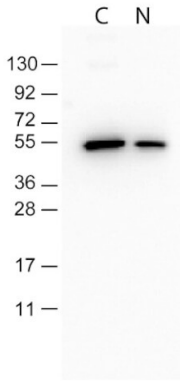
Immunofluorescence Microscopy
Active Rab32 Promotes Autophagy. A Representative immunofluorescence images of MCF7 cells stably expressing LC3-GFP and transfected with FLAG-tagged wild type (WT), dominant active (Q85L) or dominant negative (T39N) Rab32, probed for FLAG-tagged Rab32 (red) and nuclei (DAPI, blue) in parallel. Bar indicates 15 µm. Quantification of cells with > 10 LC3 puncta, indicating increased autophagy, were expressed as a percent of total cells counted. ≥ 100 cells per condition were counted in each (n = 3) independent experiment **p ≤ 0.001. Inset shows magnification of a representative area, evidencing overlap between Rab32 and LC3-GFP. Fig 1. PMID: 34743744



Immunofluorescence Microscopy
B Immunofluorescence analysis of TMX1 incorporation into LC3B-decorated structures. Images and insets show control and Rab32Q85L-transfected cells processed for anti-FLAG (blue), anti-LC3B (red) and anti-TMX1 (green) signals.
Fig 4. PMID: 34743744



Western Blot
Twenty-four (24) clones were randomly selected and grown up from glycerol stocks by inoculating 0.5mL 2xYT medium. Expression of recombinant proteins was induced by the addition of IPTG. Proteins were purified by nickel affinity chromatography 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.



Western Blot
Monoclonal Antibody to detect FLAG™ conjugated proteins detects both C terminal linked and N terminal linked FLAG™ tagged recombinant proteins by western blot.

References

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