

**Datasheet for 200-301-383S****DYKDDDDK Tag (Anti-FLAG®) Antibody****Overview**

|                      |                                                                                                    |
|----------------------|----------------------------------------------------------------------------------------------------|
| <b>Description:</b>  | Antibody for the detection of FLAG® conjugated proteins (MOUSE) Monoclonal Antibody - 200-301-383S |
| <b>Item No.:</b>     | 200-301-383S                                                                                       |
| <b>Size:</b>         | 25 µL                                                                                              |
| <b>Applications:</b> | ELISA, WB, CHIP, FC, IF, IP                                                                        |
| <b>Reactivity:</b>   | FLAG-Tag                                                                                           |
| <b>Host Species:</b> | Mouse                                                                                              |

**Product Details**

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| <b>Background:</b>   | 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. |
| <b>Synonyms:</b>     | mouse anti-FLAG™ tag, Enterokinase Cleavage Site (ECS), mouse anti-DYKDDDDK, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Host Species:</b> | Mouse                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Clonality:</b>    | Monoclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Clone ID:</b>     | 29E4.G7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Format:</b>       | IgG2a                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

## Target Details

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| <b>Reactivity:</b>         | FLAG-Tag                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Immunogen Type:</b>     | Conjugated Peptide                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Immunogen:</b>          | 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-Lys) conjugated to KLH using maleimide.                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Purity/Specificity:</b> | 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. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"> <li><a href="#">200-301-382 SDS</a></li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

## Application Details

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| <b>Tested Applications:</b>    | ELISA, WB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Suggested Applications:</b> | ChIP, FC, IF, IP (Based on references)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Application Note:</b>       | <p>Anti-FLAG has been tested by ELISA and western blot. 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.</p> |
| <b>Assay Dilutions:</b>        | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>ELISA:</b>                  | 1:20,000 - 1:100,000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>FC:</b>                     | User Optimized                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

|             |                    |
|-------------|--------------------|
| <b>IHC:</b> | User Optimized     |
| <b>WB:</b>  | 1:2,000 - 1:20,000 |

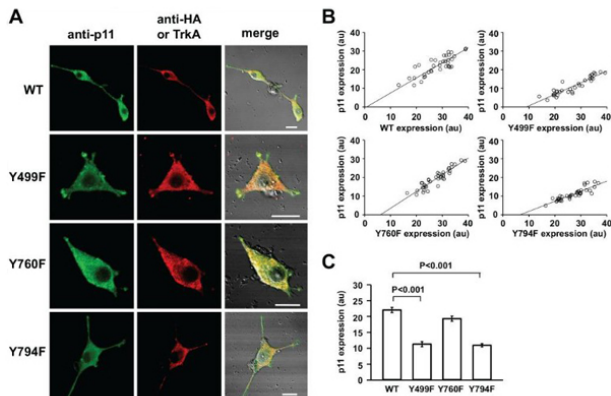
## Formulation

|                        |                                                            |
|------------------------|------------------------------------------------------------|
| <b>Physical State:</b> | Liquid (sterile filtered)                                  |
| <b>Concentration:</b>  | 1.0 mg/mL by UV absorbance at 280 nm                       |
| <b>Buffer:</b>         | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| <b>Preservative:</b>   | 0.01% (w/v) Sodium Azide                                   |
| <b>Stabilizer:</b>     | None                                                       |

## Shipping & Handling

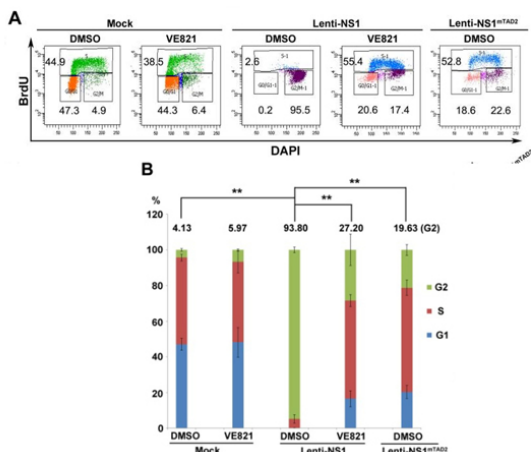
|                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Shipping Condition:</b> | Dry Ice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Storage Condition:</b>  | Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing. |
| <b>Expiration:</b>         | Expiration date is three (3) months from date of receipt.                                                                                                                                                                                                                                                                                                                                                                                                                                          |

## Images



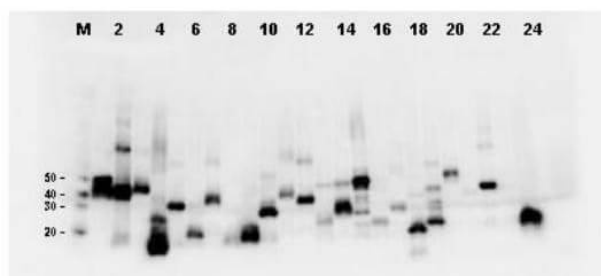
### Immunofluorescence Microscopy

Immunocytochemical analysis of signaling pathways involved in NGF-induced p11 production. (A) Confocal images of p11 and HA- or TrkA-like immunofluorescence in PC12nnr5 cells expressing wild-type (WT), Y499F-TrkA, Y760F-TrkA, or Y794F-TrkA. First and second columns represent p11- and HA- or Flag-like (p/n 200-301-383) immunofluorescence images, respectively; third, merge of DIC and fluorescence images. HA-like fluorescence was observed in cells expressing WT, Y760F-TrkA, and Y794F-TrkA, whereas TrkA-like fluorescence was observed in cells expressing Y760F-TrkA. (B) p11-like fluorescence is plotted against those of HA- (WT, Y499F, and Y794F) or TrkA-like (Y760F) fluorescence observed in the same cells. Linear regression lines are as follows—WT:  $y = -0.634 + 0.810x$ , correlation coefficient  $r = 0.823$ ; Y499F:  $y = -5.595 + 0.621x$ ,  $r = 0.924$ ; Y760F:  $y = -5.594 + 0.901x$ ,  $r = 0.929$ ; Y794F:  $y = -3.623 + 0.541x$ ,  $r = 0.888$ . (C) Summary of p11-like IR material in PC12nnr5 cells expressing WT, Y499F-TrkA, Y760F-TrkA, or Y794F-TrkA. P11-like IR material was measured over the whole cell area including protrusions or neurites. Data are mean  $\pm$  SEM; Kruskal–Wallis one-way analysis of variance on ranks (WT:  $n=38$  from three culture dishes; Y499F:  $n=33$  from two; Y760F:  $n=38$  from three; Y794F:  $n=38$  from 3). Bars are 10  $\mu$ m. Abbreviations: DIC, differential interference contrast; HA, hemagglutinin; IR, immunoreactive; NGF, nerve growth factor; PC, pheochromocytoma. Fig 5. PMID: 32886017



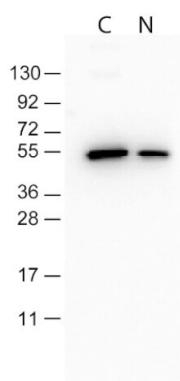
### Flow Cytometry

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



#### 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  $\mu$ L. 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

- Julian JD et al. Xyloglucan xylosyltransferase stem region mediates heterodimer formation. *Nat Commun.* (2026)
- Skotnicka, D et al. CdbA is a DNA-binding protein and c-di-GMP receptor important for nucleoid organization and segregation in *Myxococcus xanthus*. *Nature Communications* (2020)
- Matsuoka H et al. Expression of p11 and Heteromeric TASK Channels in Rat Carotid Body Glomus Cells and Nerve Growth Factor–differentiated PC12 Cells. *J Histochem Cytochem* (2020)
- Xu et al. Parvovirus B19 NS1 protein induces cell cycle arrest at G2-phase by activating the ATR-CDC25C-CDK1 pathway. *PLOS Pathogens* (2017)
- Saha et al. Human adenovirus type 5 vectors deleted of early region 1 (E1) undergo limited expression of early replicative E2 proteins and DNA replication in non-permissive cells. *PLOS One* (2017)
- Wu et al. Potassium and the K<sup>+</sup>/H<sup>+</sup> Exchanger Kha1p Promote Binding of Copper to ApoFet3p Multi-copper Ferroxidase. *Journal of Biological Chemistry* (2016)
- Turchinovich et al. Interference in transcription of overexpressed genes by promoter-proximal downstream sequences. *Scientific Reports* (2016)
- Lee et al. Transcriptional regulation of Niemann-Pick C1-like 1 gene by liver receptor homolog-1. *BMB Reports* (2015)
- Ortiz-Sandoval CG. Et al. Interaction with the effector dynamin-related protein 1 (Drp1) is an ancient function of Rab32 subfamily proteins. *Cell Logist.* (2014)

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.