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www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 600-403-383

DYKDDDDK Tag (Anti-FLAG®) Antibody Peroxidase Conjugated

Overview

Description:	Antibody for the detection of FLAG® conjugated proteins (RABBIT) Antibody Peroxidase Conjugated - 600-403-383
Item No.:	600-403-383
Size:	100 µg
Applications:	ELISA, WB, IF
Reactivity:	FLAG-Tag
Host Species:	Rabbit

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:	rabbit anti-FLAG antibody peroxidase conjugation, HRP conjugated rabbit anti-FLAG antibody, anti-Flag tagged, anti-DYKDDDDK
Host Species:	Rabbit
Conjugate:	Peroxidase (HRP)
Clonality:	Polyclonal
Format:	IgG

Target Details

Reactivity:	FLAG-Tag
Immunogen Type:	Conjugated Peptide
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-Asp-Lys) conjugated to KLH using maleimide. This antibody reacts with FLAG conjugated proteins.
Purity/Specificity:	This affinity purified 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 at either the N or C terminus. In western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins.

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF (Based on references)
Application Note:	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 amino- and carboxy- termini 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. Rockland 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000
IHC:	1:500 - 1:2,000
WB:	1:2,000 - 1:5,000

Formulation

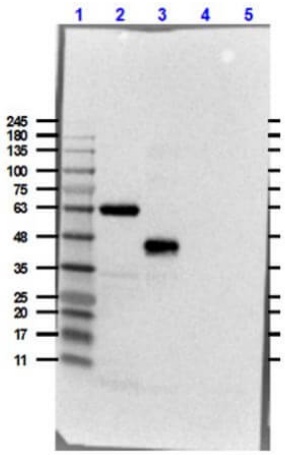
Physical State:	Lyophilized

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) Gentamicin Sulfate. Do NOT add Sodium Azide!
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

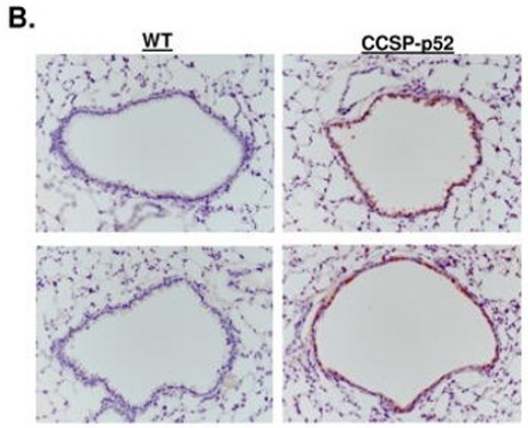
Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. 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.

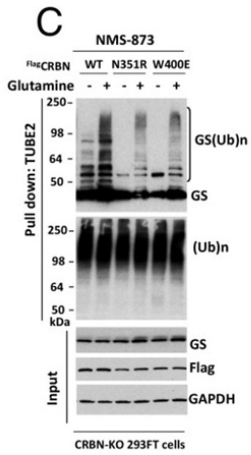
Images



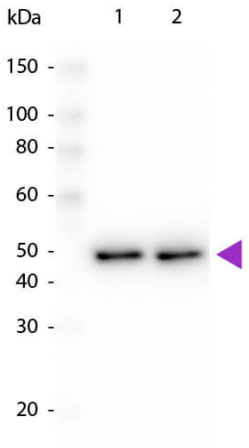
Western Blot
Western Blot of Peroxidase conjugated Rabbit Anti-DYKDDDDK same epitope as Sigma's Anti-FLAG antibody.
Lane 1: Opal Presatained Molecular Weight Marker (p/n MB-210-0500).
Lane 2: FLAG Positive Control Lysate (p/n MB-) Reduced 5µg [+].
Lane 3: 12 Epitope Tag Lysate-GST (p/n MB-) Reduced 5µg [+].
Lane 4: MBP (p/n) Reduced 0.1µg [-].
Lane 5: rGST (p/n) Reduced 0.1µg [-].
Primary Antibody: Antibody for the detection of FLAG™ HRP 1.0µg/mL overnight at 2-8°C.
Secondary Antibody: Goat Anti-Rabbit IgG HRP MX10 (p/n 611-103-122) at 1:70,000 for 30 mins at RT.
Block: Universal Block Out Buffer (p/n MB-073) 1hr RT.
Exposure 1sec.



Immunohistochemistry
Characterization of a novel transgenic mouse model of lung epithelial p52 expression. B) FLAG immunostaining of lung sections from WT and CCSP-p52 mice on dox for 1 week (20x magnification) using (p/n 600-403-383). Fig 2.
PMID: 26773153



Western Blot
(C) CRBN-KO 293FT cells stably expressing WT FlagCRBN or the indicated mutants were starved of glutamine for 24 h. Cells were pretreated with NMS-873 (10 µM) for 30 min, followed by the addition (or not) of 4 mM glutamine for 2 h. Cell lysates were fractionated on a TUBE2 resin, and both lysate samples and the bound fractions were analyzed by SDS/PAGE and immunoblotting with antibodies against GS, ubiquitin, Flag, and GAPDH. Fig 3. PMID: 28320958



Western Blot
Western blot of Peroxidase conjugated Rabbit Anti-DYKDDDDK same epitope as Sigma's Anti-FLAG antibody. Lane 1: BAP FLAG C-Term. Lane 2: BAP FLAG N-Term. Load: 50 ng per lane. Primary antibody: None. Secondary antibody: Peroxidase rabbit secondary antibody at 1:1,000 for 60 min at RT. Blocking: MB-070 for 30 min at RT. Predicted/Observed size: 49 kDa, 49 kDa for BAP FLAG. Other band(s): None.

References

- Nguyen TV et al. p97/VCP promotes degradation of CRBN substrate glutamine synthetase and neosubstrates. *Proc Natl Acad Sci USA.* (2017)
- Saxon et al. p52 Overexpression Increases Epithelial Apoptosis, Enhances Lung Injury, and Reduces Survival after Lipopolysaccharide Treatment. *Journal of Immunology* (2016)
- Benjamin et al. Epithelial-Derived Inflammation Disrupts Elastin Assembly and Alters Saccular Stage Lung Development. *The American Journal of Pathology* (2016)

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