Order online now!

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

Datasheet for 600-406-383

DYKDDDDK Tag (Anti-FLAG®) Antibody Biotin Conjugated

Overview

Description:	Antibody for the detection of FLAG® conjugated proteins (RABBIT) Antibody Biotin Conjugated - 600-406-383
Item No.:	600-406-383
Size:	100 µg
Applications:	ELISA, WB
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 antibody for the detection of FLAG™ conjugated proteins biotin conjugated, rabbit anti DYKDDDDK Biotin
Host Species:	Rabbit
Conjugate:	Biotin
Clonality:	Polyclonal
Format:	IgG
F/P Ratio:	10-20

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. To date this antibody has reacted with all amino-terminal FLAG™ tagged proteins so far tested. In western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins.

Application Details

Tested Applications:	ELISA, WB
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 carboxy or amino- terminus 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:	1:10,000 - 1:50,000
IHC:	1:1,000 - 1:5,000
WB:	1:2,000 - 1:10,000

Formulation

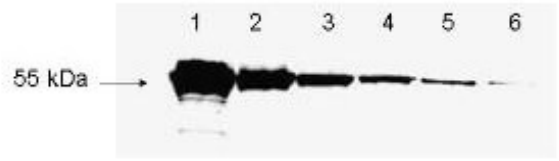
Physical State:	Lyophilized

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) 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)

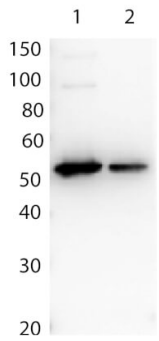
Shipping & Handling

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
Rockland's antibody to detect FLAG™ conjugated proteins is shown to detect as little as 3 ng of amino-terminal FLAG™ tagged recombinant protein by western blot. This antibody was used at a 1:1,000 dilution to detect 3-fold serial dilutions of amino-terminal FLAG™-Bacterial Alkaline Phosphatase (BAP) fusion protein (Sigma P-7582) starting at 1.0 µg of protein as shown in lanes 1-6 respectively. A 4-20% gradient gel was used to separate 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® 800 conjugated Gt-a-Rabbit IgG (H&L) (code 611-132-122) for 30 min at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.



Western Blot

Affinity Purified Antibody to detect FLAG™ conjugated proteins detects both C terminal linked and N terminal linked FLAG™ tagged recombinant proteins by western blot. This antibody was used at a dilution of 1:2,500 to detect 1.0 µg of recombinant protein containing either the FLAG™ epitope tag linked at the carboxy (C) or the amino (N) terminus of the recombinant protein. 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® 800 conjugated Gt-a-Rabbit IgG (H&L) MX10 (code 611-132-122) for 30 min at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.

Disclaimer