Antibody for the detection of FLAG™ conjugated proteins (RABBIT) - 600-401-383S

Code: 600-401-383S **Size:** 25 µL

Product Description: Antibody for the detection of FLAG™ conjugated proteins (RABBIT) - 600-401-383S

Concentration: 1.0 mg/mL by UV absorbance at 280 nm

PhysicalState: Liquid (sterile filtered)

Label	Unconjugated
Host	Rabbit
Species Reactivity	Carboxy and amino terminal linked FLAG™ tagged recombinant proteins
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Stabilizer	None
Preservative	0.01% (w/v) Sodium Azide
Storage Condition	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.
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 either the amino- or carboxy- termini of targeted proteins. This antibody has been tested by ELISA and western blotting against both the immunizing peptide and FLAGä containing recombinant proteins. Although not tested, this antibody is likely functional for immunoprecipitation, 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.
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 biochemical properties of the tagged protein. Most often, sequences encoding the epitope tag are included with the 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.
Purity And 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. In western blotting of bacterial extracts, the antibody does not cross-react with endogenous proteins.
Assay Dilutions	User Optimized
ELISA	1:90,000 - 1:250,000
Immunohistochemistry	User Optimized
WESTERN BLOT	1:2,000 - 1:10,000
IHC	User Optimized
OTHER ASSAYS	User Optimized
Expiration	Expiration date is one (1) year from date of opening.
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. Residues of glycine and cysteine were added to the carboxy terminal end to facilitate coupling. This antibody reacts with FLAG™ conjugated proteins.
General Reference	Chubet, R.G. and Brizzard, B.L. (1996) Vectors for expression and secretion of FLAG epitope-tagged proteins in mammalian cells. Biotechniques 20(1):136-141. Slootstra, J.W., et al. (1997) Identification of new tag sequences with differential and selective recognition

properties for the anti-FLAG monoclonal antibodies M1, M2 and M5. Mol. Divers. 2(3):156-164.

Robeva, A.S., et al. (1996) Double tagging recombinant A1- and A2A-adenosine receptors with hexahistidine and the FLAG epitope. Development of an efficient generic protein purification procedure. J. Biochem. Pharmacol. 51(4):545-555.

Fulton, J.E. et al. (1995) Functional analysis of avian class I (BFIV) glycoproteins by epitope tagging and mutagenesis in vitro. Eur. J. Immunol. 25(7):2069-2076.

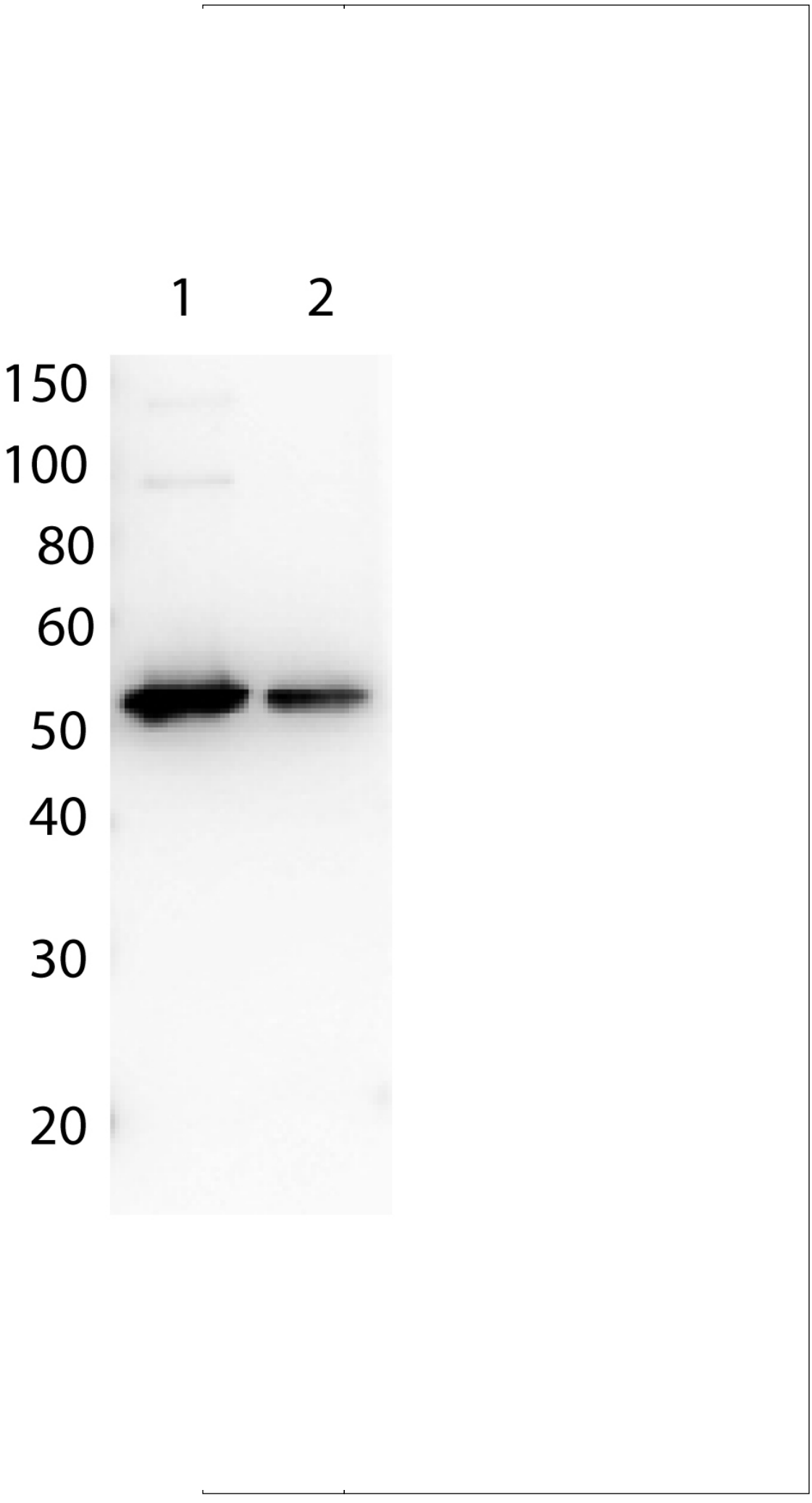
Related Products

200-301-268	Anti-AKT pS473 (MOUSE) Monoclonal Antibody - 200-301-268
600-401-383	Antibody for the detection of FLAG™ conjugated proteins (RABBIT) - 600-401-383
610-4302	Anti-MOUSE IgG (H&L) (RABBIT) Antibody Peroxidase Conjugated - 610-4302
611-1302	Anti-RABBIT IgG (H&L) (GOAT) Antibody Peroxidase Conjugated - 611-1302

Related Links

Images

1	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 1:1,000 to detect 0.1 ?g of recombinant protein containing either the FLAG? epitope tag linked at the carboxy (C), Lane 2, or the amino (N), Lane 1, 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 overnight at 4°C followed by washes and reaction with a 1:40,000 dilution of HRP conjugated Gt-a-Rabbit IgG (H&L) MX10 (code 611-103-122) for 30 min at room temperature. Bio-Rad's VersaDoc® 4000 MP Imaging System was used to process the image. Other detection systems will yield similar results


1
2
150
100
80
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50
40
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2	Western Blot of Rabbit anti-FLAG antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-363).Lane 3: CHO/K1 Lysate (p/n W07-000-357).Lane 4: MDA-MB-231 (p/n W09-001-GK6).Lane 5: A431 Lysate (p/n W09-000-361).Lane 6: Jurkat Lysate (p/n W09-001-370).Lane 7: NIH/3T3 Lysate (p/n W10-000-358).Lane 8: E-coli HCP Control (p/n 000-001-J08).Lane 9: FLAG Positive Control Lysate (p/n W00-001-383Lane 10: Red Fluorescent Protein (p/n 000-001-379).Lane 11: Green Fluorescent Protein (p/n 000-001-215).Lane 12: Glutathinoe-S-Transferase ProteinLane 13: Maltose Binding Protein (p/n 000-001-385).Load: 10 µg of lysate or 50ng of purified protein per lane.Primary antibody: FLAG antibody at 1ug/mL overnight at 4C.Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT.Blocking Buffer: 1% Casein-TTBS for 30 min at RT.Predicted/Observed size: 55 kDa for FLAG.
	

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.