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Certificate of Analysis

Product: IRDye™ 800CW Conjugated Affinity Purified Antibody for the detection of FLAG™ conjugated proteins (Rabbit)

Code: 600-431-383

Lot # 12875

Size: 100 µg

Physical State: Lyophilized

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Label: IRDye™ 800CW (MW 1166.2)

Fluorochrome/Protein Ratio: 1.1 moles IRDye™ 800CW /mole of Rabbit IgG

Absorption Wavelength: 774 nm (in PBS)

Emission Wavelength: 800 nm

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: 10 mg/ml BSA IgG and Protease free

Preservative: 0.01% (w/v) Sodium Azide

Background Information: 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.

Recommended Dilution(s):	LI-COR Odyssey® BLOT	1:2,500 - 1:5,000
	LI-COR In-Cell Western®	User Optimized
	OTHER APPLICATIONS	User Optimized

Application(s): Fluorescence technology is widely used to detect proteins. However, many common visible fluorophores often result in considerable background fluorescence in the visible range. Visible fluorophores are rarely used for membrane-based protein detection because of this high background. IRDye™ 800 antibody and reagent conjugates are specifically designed for protein detection methods that use longer-wavelength, near-infrared (IR) fluorophores to visualize proteins in western blotting and other applications. Very low background fluorescence in the IR range provides for a much higher signal-to-noise ratio than visible fluorophores. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. IRDye™ 800 conjugates are optimized for the Odyssey® Infrared Imaging System developed by LI-COR. IRDye™ 800 conjugates are also suitable for immunofluorescence microscopy using commercially available excitation/emission filters in the 780nm/820nm range. Dual simultaneous labeling in western blots or microscopy is achieved when IRDye™ 800 conjugates are used in conjunction with Cy5.5™ conjugates. IRDye™ 800 conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging. IRDye™ 800CW conjugates are specifically designed for LI-COR's In-Cell Western assay for the Odyssey® Infrared Imaging System. IRDye™ 800CW conjugates can be used for western blotting applications, but the fluorochrome/protein ratio is not optimal for this application so detection sensitivity may be reduced. The In-Cell Western assay is a faster method for analyzing signal transduction pathways. In less time than a gel-based Western, you can quantify proteins in a 96- or 384-well microplate. In-Cell Westerns use infrared dye-labeled secondary antibodies to directly label proteins in fixed cultured cells, and quantify total fluorescence from each well. Time consuming and error-prone steps such as lysate preparation, gel loading and electrophoresis, and membrane transfer are eliminated with In-Cell Westerns. Simultaneous, two-target detection enables quantitative and accurate measurement of abundance or phosphorylation of one target by normalization against another target. Near-infrared probes yield high sensitivity for measuring small changes in protein amount or modification. Direct detection of proteins in their cellular context eliminates artifacts caused by cell lysis. The fast, microplate-based assay

eliminates lysates, gels, and membranes required for conventional Western blots. The In-Cell Western assay is a moderate throughput method, ideal for screening cell treatments or drug candidates for their effects on target proteins. Recommended plates for In-Cell Westerns are Nunc-Nalgene (p/n 167008) for 96-well format and Falcon (BD Biosciences p/n 353961) for 384-well format. Primary and secondary antibodies should be validated by Odyssey western blotting *before* performing the In-Cell Western assay to confirm specificity and performance in the infrared system. 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- terminus of targeted proteins. No reactivity is seen against fusion proteins containing the epitope tag at the carboxy- terminus. 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.

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). Centrifuge product if not completely clear after standing at room temperature. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of restoration.

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. 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.

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.

Conjugation Reference: LI-COR Biosciences, Lincoln, NE.

Related Products:

#600-401-381	Affinity Purified Anti-Myc TAG (Rabbit)
#600-401-382	Affinity Purified Anti-6X His TAG (Rabbit)
#600-401-383	Affinity Purified Antibody for the detection of FLAG™ conjugated proteins (Rabbit)
#600-431-383	IRDYE800CW™ Conjugated Affinity Purified Antibody for the detection of FLAG™ conjugated proteins (Rabbit)
#600-432-383	IRDYE800™ Conjugated Affinity Purified Antibody for the detection of FLAG™ conjugated proteins (Rabbit)
#600-401-384	Affinity Purified Anti-HEMAGGLUTININ (Rabbit)
#200-101-150	IgG Fraction of Anti-LUCIFERASE (GOAT)
#600-101-200	Affinity Purified Anti-GST (GOAT)
#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	MaxTag™ Anti-RABBIT IgG Kit for Immunoblotting

USDA Certification: 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.

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