

Certificate of Analysis

Product: IRDye™ 800CW Conjugated Affinity Purified anti-Green Fluorescent Protein (*Aequorea victoria*) [Goat] Minimum Cross Reactivity to Human, Mouse and Rat Serum Proteins

Code: 600-131-215

Lot # 18382

Size: 500 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: 1.6 moles IRDye™ 800CW/mole of Goat IgG

Absorption Wavelength: 774 nm (in PBS)

Emission Wavelength: 800 nm

Buffer: 0.02 M Potassium Phosphate, 0.15 M NaCl, pH 7.2

Preservative: 0.01% (w/v) Sodium Azide

Stabilizer: 10 mg/ml Bovine Serum Albumin (BSA) IgG and Protease free

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.

Recommended Dilution(s): This product was tested by immunoblot using GFP spotted to nitrocellulose membrane. A 1:2,500 dilution is sufficient to detect 25-50 pg of immobilized GFP containing protein. In general, a 1:800 dilution is sufficient to detect signal in the In-Cell Western assay. Researchers should determine optimal titers for their targets and cell types.

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.5 ml of deionized water (or equivalent). 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 date is one (1) year from date of restoration.

Purity: This product was prepared from monospecific antiserum by immunoaffinity chromatography using Green Fluorescent Protein (*Aequorea victoria*) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Goat Serum and purified and partially purified Green Fluorescent Protein (*Aequorea victoria*) Serum. No reaction was observed against Human, Mouse or Rat serum proteins.

Immunogen: The immunogen is a GST- Green Fluorescent Protein (GFP) fusion protein corresponding to the full length amino acid sequence (246aa) derived from the jellyfish *Aequorea victoria*.

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

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