

**Certificate of Analysis****Product:** IRDye™ 800CW Conjugated Affinity Purified anti-Fluorescein [Goat]**Code:** 600-131-096**Lot #** 16201**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:** 1.5 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 Sodium Chloride, pH 7.2**Stabilizer:** 10 mg/ml BSA IgG and Protease free**Preservative:** 0.01% (w/v) Sodium Azide

**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 and IRDye™ 700DX 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.

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 will 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. Specificity and performance in the infrared system should be validated for primary and secondary antibodies by Odyssey Western blotting *before* performing the In-Cell Western assay.

|                                 |                                |                 |
|---------------------------------|--------------------------------|-----------------|
| <b>Recommended Dilution(s):</b> | <b>LI-COR Odyssey® BLOT</b>    | Not Recommended |
|                                 | <b>LI-COR In-Cell Western®</b> | 1:800           |
|                                 | <b>OTHER APPLICATIONS</b>      | User Optimized  |

**Storage Conditions:** Store vial at 4° C prior to restoration. Restore with 0.5 ml of deionized water (or equivalent). This product is stable for several weeks at 4° C as an undiluted liquid. 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. 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 Fluorescein IgG 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 Fluorescein conjugated Bovine Serum Albumin.

**Immunogen:** Fluorescein conjugated to Goat IgG**Conjugation Reference:** LI-COR Biosciences, Lincoln, NE.

**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.

**Note:** This material is subject to proprietary rights and is sold under license from LI-COR, Inc. This product is licensed for sale only for 'research-use' only. There is no implied license hereunder for any commercial use. IRDye is a trademark of LI-COR, Inc. COMMERCIAL USE shall include:

1. Resale, lease, license or other transfer of the material or any material derived or produced from it.
2. Resale, lease, license or other grant of rights to use this material or any material derived or produced from it.
3. Use of this material to perform services for a fee for third parties.

If you require a commercial license to use this material and do not have one, return this material, unopened to Rockland Inc. PO BOX 326, Gilbertsville, PA and money paid for the material will be refunded.