

### Certificate of Analysis

**Product:** Blocking Buffer for Near Infra Red Fluorescent Western Blotting

**Code:** MB-070

**Lot #** 20085

**Size:** 500 ml

**Physical State:** Liquid (sterile filtered)

**Buffer Composition:** This buffer was meticulously prepared using ultra pure reagents dissolved in pharmaceutical grade water (WFI) and consists of a proprietary protein formulation in TRIS buffered saline at pH 7.6 with thimerisol added as an antimicrobial agent.

**Application(s):** This blocking buffer is specifically designed for western blotting using IRDye® conjugated antibodies. Pure nitrocellulose membrane is recommended for maximum performance. Other membranes, such as PVDF or nitrocellulose embedded in a support can be used, but may generate elevated backgrounds. Protein should be transferred from gel to membrane using standard protocols. This buffer can be used for membrane blocking and to dilute both primary and secondary antibodies. See [www.rockland-inc.com](http://www.rockland-inc.com) for specific protocols.

**Background Information:** 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, IRDye® 700DX, Alexa Fluor® 680 and Cy5.5™ 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. IRDye® 800 and IRDye® 700DX fluoresce outside the range of most autofluorescence and therefore specific signal is sharply evident from any background giving the best possible signal-to-noise ratio. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. 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 IRDye® 700 or Cy5.5™ conjugates. IRDye® 800 and IRDye® 700DX conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging. Once reacted with the membrane and dried, IRDye® 800 and IRDye® 700DX conjugated antibody-protein complexes are very stable, and membranes can be stored protected from light, re-washed and/or rescanned. Blocking Buffer for Fluorescent Western Blotting is specifically formulated to achieve superior reproducible western blotting images using this system.

**Recommended Dilution(s):** Optimum performance is achieved using this product undiluted. However, this blocking solution can often be diluted 1:1 in TBS without a significant loss of performance.

**Storage Conditions:** Store container at 4° C before opening. **DO NOT FREEZE.** Expiration date is six (6) months from date of receipt if properly handled and stored.

**Sterilization:** This product was aseptically filtered through a Millipore 0.22-micron filter into clean, pre-sterilized containers. The product was tested on trypticase soy agar for 24 hours, 48 hours and 72 hours and was found to be negative for bacteria.

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