NewBlot™ IR Stripping Buffer Pack Insert

Components

- NewBlot IR Stripping Buffer (928-40028)
- Store: Room Temperature

  Make 1X solution as needed and store at room temperature.

Applications

Nitrocellulose and PVDF membranes used for near-infrared Western blot detection can be stripped and re-probed using NewBlot IR Stripping Buffer. The stripping buffer is a robust but gentle, non-hazardous formulation for stripping primary and secondary antibodies from Western blots to enable a membrane to be reprobed. NewBlot IR is optimized for effective stripping of primary antibodies and IRDye® labeled secondary antibodies and may be used for removal of other dye-labeled secondary antibodies.

Required Materials

- Buffer, such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) with 0.1% Tween® 20
- Primary and secondary antibodies for Western blot experiments
- Odyssey® Imager or imaging system with similar excitation and emission capabilities

Protocol

Blots may be stored in TBS or PBS until the stripping procedure can be performed. Do not allow blots to dry out.

Note: *Performance of stripping buffer may be compromised for protein amounts over 30 µg.*

1. Before you begin, inspect the NewBlot Stripping Buffer for precipitated material.

   If precipitated material is apparent, gently swirl the bottle in a warm water bath until precipitate is dissolved.

2. Prepare 1X Working Solution by mixing one part NewBlot Stripping Buffer with four parts laboratory-grade water.

3. Place the blot in 1X Working Solution and incubate for 15 minutes at room temperature on an orbital shaker. Use a sufficient volume of Working Solution to ensure that the blot is completely submerged.

   Note: *Use the Working Solution at room temperature. Higher temperatures and higher concentrations lead to lower stripping efficiency.*

4. Remove the blot from the Working Solution and wash in buffer at room temperature for 5 minutes on an orbital shaker.

   Repeat the wash two times.

5. Proceed to blocking step.

Imaging

Some residual signal may appear on a membrane imaged after stripping. To evaluate the relative amount of signal remaining, compare the original image to the second image in the Image Gallery of LI-COR® Acquisition Software or Empiria Studio® Software or in Image Studio™ Software.

Each imager and scanner's imaging parameters and protocols vary slightly. Please reference the imager or scanner's user manual for more information.

Note: *Step-by-step image acquisition workflows are available in LI-COR Acquisition Software. Use the recommended imaging parameters to help optimize acquisition for comparison purposes.*

For LI-COR Acquisition Software and Empiria Studio, match the images' display settings in the Image Gallery.

1. Open the first image in the Image Gallery and view its values in the display settings values (e.g., Min and Max).
2. Set the image display settings (e.g., Min and Max) to the same values as the second image.

For Image Studio, compare the images with their lookup tables linked. More detailed instructions can be found by searching the Image Studio Help for the phrase "link lookup".

1. Select the images to compare in the Images Table and apply a selection filter to the selected images by clicking CTRL + J.
2. Click **Link** above the Brightness and Contrast sliders.

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LI-COR®

LI-COR Biosciences
4647 Superior Street
Lincoln, NE 68504
Phone: +1-402-467-0700
Toll free: 800-645-4267
biosales@licor.com
licor.com/bio

Regional Offices

LI-COR Biosciences GmbH
Siemensstraße 25A
61352 Bad Homburg
Germany
Phone: +49 (0) 6172 17 17 771
bio-eu@licor.com

LI-COR Biosciences UK Ltd.
St. John's Innovation Centre
Cowley Road • Cambridge
CB4 0WS • United Kingdom
Phone: +44 (0) 1223 422104
bio-eu@licor.com

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