INSTRUCTIONS

Restore Fluorescent Western Blot Stripping Buffer

62299 62300

2183.0

Number	Description
62299	**Restore Fluorescent Western Blot Stripping Buffer (5X)**, 20 ml, sufficient for 10 (8 × 10 cm) blots
62300	**Restore Fluorescent Western Blot Stripping Buffer (5X)**, 100 ml, sufficient for 50 (8 × 10 cm) blots

Storage: Upon receipt store at room temperature.

Introduction

The Thermo Scientific Restore Fluorescent Western Blot Stripping Buffer provides a robust but gentle method of removing the primary and secondary antibodies from fluorescent Western blots, enabling reprobing of a previously probed membrane. Reprobing a stripped blot saves time and cost when samples are in limited quantities, when the same sample requires analysis by different antibodies, or when optimization is required. Traditional stripping methods may adversely alter the proteins on the membrane or use conditions that are effective for only low-affinity antibody-antigen interactions. The Restore Fluorescent Western Blot Stripping Buffer is used at room temperature to quickly and effectively strip most high-affinity antibodies.

Additional Materials Required

- Western blot detected with fluorescent dye-labeled antibodies

  Note: Use low-fluorescence PVDF membrane (Product No. 22860) and a blocking buffer optimized for fluorescent Western blotting (e.g., Thermo Scientific SEA BLOCK Blocking Buffer, Product No. 37527)

- Wash buffer such as Tris-buffered saline (TBS, Product No 28376) containing 0.05% Tween®-20 Detergent (Product No. 28320)
- Tris-buffered saline (TBS, Product No 28376)
- Primary and/or secondary antibodies labeled with appropriate fluorescent dye(s). The following secondary antibodies have been used successfully:
  - Goat anti-Mouse IgG, DyLight 680 Conjugated (Product No. 35519)
  - Goat anti-Mouse IgG, DyLight 800 Conjugated (Product No. 35521)
  - Goat anti-Rabbit IgG, DyLight 680 Conjugated (Product No. 35568)
  - Goat anti-Rabbit IgG, DyLight 800 Conjugated (Product No. 35571)

Protocol for Stripping a Fluorescent Western Blot

Notes:

- When performing multiple strips and re-probing for different antigens, probe for the low-abundance proteins first.
- Do not re-use stripping buffer. Fluorescent dye in used stripping buffer will contribute to background when reprobing.
- After initial probe, blots may be stored in TBS at 4°C until performing the stripping procedure. Do not allow blots to dry.

1. Dilute the Restore Fluorescent Western Blot Stripping Buffer to 1X by mixing one part stripping buffer with four parts ultrapure water. Each 8 × 10 cm membrane requires 10 ml of 1X stripping buffer.

   Note: The concentration of the stripping buffer might require optimization.

2. Place the blot into the diluted stripping buffer and incubate for 10 to 20 minutes at room temperature with constant shaking.
3. Immediately rinse membrane with ultrapure water three times. Wash membrane three times with Wash Buffer (3 × 5 minutes). Rinse and store membrane in TBS. Do not allow the blot to dry.
4. To verify sufficient antibody has been removed, image the membrane for fluorescent signal. After determining that the membrane is sufficiently stripped, proceed to the second probing experiment.

   Note: If signal remains, return to step 1, stripping for an additional 5-10 minutes. Some antibody/antigen systems require longer incubation times or a higher stripping buffer concentration (e.g., dilute stripping buffer 1:2 or use it neat). Optimize stripping times and buffer concentration to ensure complete removal of antibodies while minimizing damage to the antigen.

Troubleshooting

Problem	Possible Cause	Solution
Background after stripping	Insufficient blocking	Typically, re-blocking the membrane after stripping is not necessary; however, if there is excessive background, block membrane for an additional 30 minutes
	Scanner settings incorrectly adjusted	Adjust settings and re-image
Loss of signal or no signal after stripping and re-probing	Antigen is not present or is in low abundance	Prepare a new blot and probe for low-abundance antigens first
	Antibody or conjugate concentrations are too low	Increase concentration of primary antibody or the conjugated secondary antibody
Signal obtained after stripping	Extremely high-affinity antigen-antibody interaction	Increase incubation time of stripping step or use a higher concentration of stripping buffer

Related Thermo Scientific Products

22860 **Low-Fluorescence PVDF Transfer Membrane**

22855 **DyLight 680/800 Western Blotting Kit**

35501-35521 **Goat Anti-Mouse IgG DyLight Dye-labeled Conjugates**

35551-35571 **Goat Anti-Rabbit IgG DyLight Dye-labeled Conjugates**

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.