Thermo Fish

SCIENTIFIC

Restore[™] Fluorescent Western Blot Stripping Buffer

Doc. Part No. 2162183 Pub. No. MAN0011688 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Thermo Scientific[™] Restore[™] Fluorescent Western Blot Stripping Buffer provides a robust but gentle method for removing primary and secondary antibodies from fluorescent western blots, enabling reprobing of a previously probed membrane. Restore[™] Fluorescent Western Blot Stripping Buffer allows for reprobing thus saving time and costs when samples are in limited quantities, when the same sample requires analysis by different antibodies, and when optimization is required. Traditional stripping methods can either adversely alter the proteins on the membrane or use conditions that are effective for only low-affinity antibody-antigen interactions. Restore[™] Fluorescent Western Blot Stripping Buffer can be used at room temperature to quickly and effectively strip most high-affinity antigenantibody interactions. Restore[™] Fluorescent Western Blot Stripping Buffer is optimized for use with low fluorescence, PVDF-probed membranes.

Contents and storage

Product	Cat. No.	Amount	Storage
Restore [™] Fluorescent Western Blot Stripping Buffer	62299	20 mL	Room temperature
	62300	100 mL	

Procedural guidelines

- Restore[™] Fluorescent Western Blot Stripping Buffer is supplied in a 5X format and it is recommended to dilute to 1X prior to use. Other concentrations may be beneficial for specific systems.
- Do not re-use stripping buffer. Fluorescent dye in used stripping buffer can contribute to background when reprobing.
- Blots may be stored in TBS at 4°C until the stripping procedure can be performed.
- Restore[™] Fluorescent Western Blot Stripping Buffer <u>will not dissociate</u> interactions between a biotinylated target protein and avidinconjugated probes.
- When performing multiple strippings and reprobings for different antigens, it is recommended to probe for low-abundant proteins first.
- Reblocking the membrane is recommended after stripping.

Strip western blots and test effectiveness of procedure

- 1. Dilute the Restore[™] Fluorescent Western Blot Stripping Buffer to 1X by mixing one part stripping buffer with four parts ultrapure water.
- 2. Add sufficient 1X Restore[™] Fluorescent Western Blot Stripping Buffer to cover the blot and incubate for 10 to 20 minutes at room temperature with constant shaking.
- 3. Immediately rinse membrane with ultrapure water.
- 4. Wash membrane 3 times for 5 minutes each in wash buffer (e.g., TBS) with shaking. Rinse and store membranes in TBS until reprobing.



5. Test the effectivenss of stripping to verify sufficient antibody has been removed, image the membrane for fluorescent signal.

Note: If signal remains, strip for 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 neat). Optimize stripping times and buffer concentration to ensure complete removal of antibodies while minimizing damage to the antigen.

6. After determining that the membrane is properly stripped, the second immunoprobing experiment may be performed.

Related products

Table 1 Stripping buffers for western blotting

Product	Select when	Available formats
Restore [™] Western Blot Stripping Buffer	Primary antibody is susceptible to stripping buffers	30 mL (Cat. No. 21062)
		500 mL (Cat. No. 21059)
		5 L (Cat. No. 21063)
Restore [™] PLUS Western Blot Stripping Buffer	Removing high affinity antibodies	30 mL (Cat. No. 46428)
		500 mL (Cat. No. 46430)
Restore [™] Fluorescent Western Blot Stripping	Removing fluorescent-labeled antibodies	20 mL (Cat. No. 62299)
Buffer		100 mL (Cat. No.62300)

Table 2 Additional products

Products	Learn more
ECL Kits	thermofisher.com/chemisubstrates
Western blot blocking buffers	thermofisher.com/blockingbuffers
Western blot transfer equipment and supplies	thermofisher.com/transfer
Western blot imaging and analysis	thermofisher.com/westernimaging

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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Revision	Date	Description
B.0	13 December 2021	Updated content and move to CCMS
A.0	17 October 2015	New document

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