Restore™ PLUS Western Blot Stripping Buffer

Doc. Part No. 2161959 Pub. No. MAN0011594 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™ PLUS Western Blot Stripping Buffer provides a robust but gentle method for removing primary and secondary antibodies from Western blots that were detected with chemiluminescent substrates. Restore™ PLUS Western Blot Stripping Buffer allows for membrane 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™ PLUS Western Blot Stripping Buffer can be used to quickly and effectively strip most high-affinity antigen-antibody interactions. Restore™ PLUS Western Blot Stripping Buffer can be used with both nitrocellulose and PVDF-probed membranes.

Contents and storage

Product	Cat. No.	Amount	Storage
Restore™ PLUS Western Blot Stripping Buffer	46428	30 mL	Room temperature
	46430	500 mL	

Procedural guidelines

- Restore™ PLUS Western Blot Stripping Buffer is supplied in ready-to-use format. It is not recommended to dilute the buffer.
- Blots may be stored in PBS or TBS at 4°C until the stripping procedure can be performed.
- Restore™ PLUS Western Blot Stripping Buffer will not dissociate interactions between a biotinylated target protein and avidin-conjugated probes.
- Stripping fluorescent western blots is not recommended with Restore™ PLUS Western Blot Stripping Buffer as results can be inconsistent. It is recommended to use Restore™ PLUS Western Blot Stripping Buffer for fluorescent applications.
- 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. Wash blot once for 5 minutes in wash buffer (PBS or TBS) to remove the chemiluminescent substrate.
2. Add sufficient Restore™ PLUS Western Blot Stripping Buffer to cover the blot and incubate for 5 to 15 minutes at 37 °C with gentle shaking.

   Note: Optimization of both incubation time and temperature is essential for best results. For some antibodies, room temperature incubation is sufficient. However, high-affinity antibodies or saturated blots (excess secondary antibody) may require incubation for an additional 5 to 10 minutes at 37°C.
3. Remove the blot from the Restore™ PLUS Western Blot Stripping Buffer and wash 2 times for 5 minutes each in TBS or PBS with gentle shaking.

4. Test the effectivenss of stripping as follows:

   To test for complete removal of the alkaline phosphatase (AP) or horseradish peroxidase (HRP) label (e.g., secondary antibody), incubate the membrane with new chemiluminescent substrate. If no signal is detected using a 5-minute exposure, the AP or HRP conjugate has been successfully removed from the antigen or primary antibody.

   To test for complete removal of the primary antibody, incubate the membrane with the AP or HRP-labeled secondary antibody, followed by 3, 5-minute washes in wash buffer. Incubate the membrane with new chemiluminescent substrate. If no signal is detected with a 5-minute exposure, the primary antibody has been successfully removed from the antigen.

5. If signal is detected with either test in step 4, incubate blot in Restore™ PLUS Western Blot Stripping Buffer for an additional 5 to 15 minutes and repeat step 2 and step 3.

   Note: Some antigen/antibody systems require increased temperature and/or longer incubation times to strip them fully. Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.

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

7. Block the membrane in blocking buffer before continuing with the second immunoprobing experiment.

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 Buffer	Removing fluorescent-labeled antibodies	20 mL (Cat. No. 62299) 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**.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
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 history: Pub. No. MAN0011594

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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