Signal clear stripping buffer

Instructions for Use

Introduction

Product code

RPN2238

Important

Read these instructions carefully before using the product.

Intended use

The product is intended for research use only, and not to be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the *Safety Data Sheets*.

Components

The product is comprised of one 250 mL vial of stripping buffer, sufficient for ten small format blots.

Storage

Store the product at room temperature.

Expiry

Refer to the label on the product packaging for expiry date.

Background

Product overview

Signal clear stripping buffer is a buffer used to remove primary and secondary antibodies from membranes that have already been blocked, probed, and detected. The product is suitable for both PVDF and nitrocellulose membranes and all chemiluminescent and fluorescent substrates. After stripping, the Western blot can be probed again with different antibodies, as the target proteins on the blots remain undamaged.

Considerations when stripping

- Do not store Western blots dry. Before stripping, the membranes must be stored in washing buffer.
- The stripping protocol removes some of the proteins immobilized on the membrane. Therefore, it is not recommended to make quantitative comparisons of the target proteins that are probed before and those probed after stripping. In addition, a membrane that has been stripped must not be probed to show the absence of proteins.
- When detecting several proteins in sequence, begin with the proteins from which a weaker signal is expected.

Protocol

Required materials

The following materials must be supplied by the user to strip the membrane with Signal clear stripping buffer.

- Membrane that has been blocked, probed, and detected.
- Washing buffer
- Orbital shaker

The following materials are optional, if a secondary antibody test is needed. This test can be used to determine stripping efficiency or optimize the stripping time and temperature.

- New chemiluminescent working solution or fluorescent secondaries
- Primary antibody towards the protein of interest
- HRP-labeled secondary antibody
- CCD imaging system

Strip the membrane

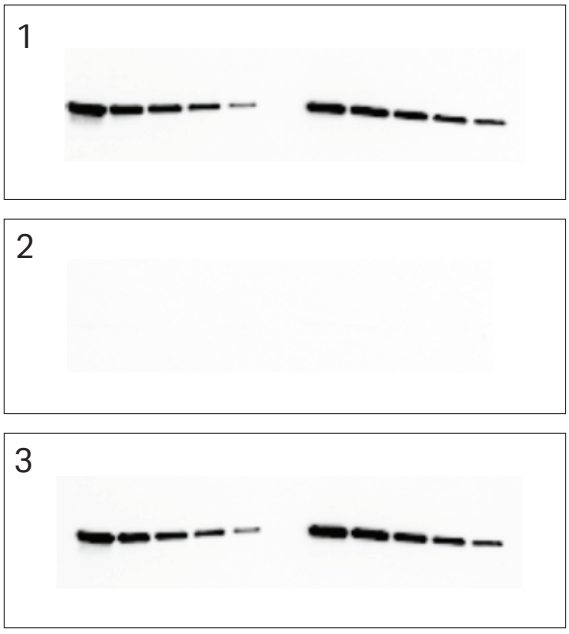
Follow the steps below to strip the membrane.

Note: *A membrane can be stripped and reprobed several times. However, additional rounds of stripping and reprobing can result in:*

- *longer exposure times required*
- *a more sensitive chemiluminescence substrate required*
- *a decreased signal*

Step	Action
1	Submerge the membrane in approximately 25 mL of stripping buffer.
2	Incubate the membrane with gentle mixing on an orbital shaker for 20 minutes at room temperature.
3	Remove the membrane from the stripping buffer and wash in washing buffer for 5 minutes.
4	Optional: Confirm the removal of the primary and secondary antibody as follows: **a.** Incubate the membrane with the HRP-labeled or fluorescent secondary antibody. **b.** Wash the membrane using the washing buffer according to the probing protocol used.

Step	Action
	c. Incubate the membrane with the new chemiluminescent working solution or scan it for fluorescence.
	d. Use an imaging system or scanner with the same settings as used in the first detection to analyze the membrane.
	e. Check that no signal is detected.
	Result: Lack of signal indicates that the primary antibody has been completely removed from the protein. The images below show an example of the original membrane (1), stripped membrane with no signal detected (2), and same membrane reprobed for the same protein with the same exposure time and contrast settings (3).



It is not necessary to reblock the membrane after stripping, but reblocking might be required in some applications.

Troubleshooting

Problem	Possible cause	Action
Bands are detected after stripping	Affinity between bound protein and antibody is high	Optimize the stripping time and temperature to ensure complete removal of antibodies while preventing damage to the membrane-bound antigen.

Related products

For more information on products, pack size, and information on additional products, visit *cytiva.com/westernblotting*.

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