INSTRUCTIONS

Restore™ Plus Western Blot Stripping Buffer

46428 46430 46431

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Number	Description
46428	**Restore Plus Western Blot Stripping Buffer,** 30mL
46430	**Restore Plus Western Blot Stripping Buffer,** 500mL
46431	**Restore Plus Western Blot Stripping Buffer,** 5L

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Introduction

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 reprobing 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 either adversely alter the proteins on the membrane or use conditions that are effective for only low-affinity antibody-antigen interactions.[1,2] Restore Plus Western Blot Stripping Buffer can be used at room temperature to quickly and effectively strip most high-affinity antigen-antibody interactions.

Additional Materials Required

- Western blot, previously blocked, probed and detected with a chemiluminescent substrate
- Wash Buffer such as Tris-buffered saline (TBS, Product No. 28376) or phosphate-buffered saline (PBS, Product No. 28372) containing 0.05% Tween®-20 (Product No. 28320)
- Primary and secondary antibodies
- Film or CCD camera for detecting the chemiluminescent signal

Protocol for Stripping an Immunoblot

Notes:

- When performing multiple strips and reprobing for different antigens, probe for the low-abundant proteins first.
- Restore Plus Western Blot Stripping Buffer will not dissociate interactions between a biotinylated target protein and avidin-conjugated probes.
- Stripping and reprobing fluorescent Western blots is not recommended because results are typically inconsistent.

1. Wash blots in Wash Buffer to remove the chemiluminescent substrate. Blots may be stored in PBS or TBS at 4°C until the stripping procedure can be performed.
2. Place the blot in Restore Plus Western Blot Stripping Buffer and incubate for 5-15 minutes at room temperature. Use a sufficient volume to ensure that the blot is completely wetted (i.e. approximately 20mL for an 8 × 10cm blot).

   Note: Optimization of both incubation time and temperature is essential for best results. In general, high-affinity antibodies require at least 15 minutes and may require incubation at 37°C.
3. Remove the blot from the Restore Plus Western Blot Stripping Buffer and wash in Wash Buffer.
4. Block membrane for 30-60 minutes at room temperature or overnight at 2-8°C.

5. Test for removal of the antibodies as follows:
   - To test for complete removal of the enzyme conjugate (e.g., secondary antibody), incubate the membrane with new chemiluminescent substrate working solution and expose blot to film. If no signal is detected using a minimum 5 minute exposure, the enzyme 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 enzyme conjugate and then wash in Wash Buffer. Incubate in new chemiluminescent substrate working solution and expose to film. If no signal is detected with a 5 minute exposure, the primary antibody has been successfully removed from the antigen.
6. If signal is detected with either test in Step 5, return to Step 1, stripping for an additional 5-15 minutes. Some antigen/antibody systems require increased temperature and/or longer incubation times. Optimize stripping time and temperature to ensure complete removal of antibodies while minimizing damage to the antigen.
7. After determining that the membrane is properly stripped, the second immunoprobing experiment may be performed.

   Note: Blot may be stripped and reprobed several times but might require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobings might result in decreased signal if the antigen is labile in Restore Plus Western Blot Stripping Buffer. Analysis of the individual system is required.

Troubleshooting

Problem	Possible Cause	Solution
Background after stripping and subsequent detection	Not sufficiently blocked after stripping	Optimize blocking conditions
	Antibody concentrations are too high	Strip again and reprobe using more dilute antibody concentrations
Loss of signal or no signal after stripping and reprobing	Antigen is not present or in low abundance	Prepare a new blot and probe for low-abundant antigens first
	Protein or antibody concentrations were too low	Increase antibody concentrations
		Load more protein in the gel
Signal obtained after stripping	Extremely high-affinity antigen-antibody interaction	Increase incubation time and temperature

Related Thermo Scientific Products

46640 **SuperSignal Western Blot Enhancer,** 500mL kit, sufficient for 25 mini blots or 2000cm^2

46641 **SuperSignal Western Blot Enhancer,** 50mL kit, sufficient for 2 mini blots or 160cm^2

32106 **Pierce® ECL Western Blotting Substrate**, 500mL

34080 **SuperSignal® West Pico Chemiluminescent Substrate**, 500mL

34075 **SuperSignal West Dura Chemiluminescent Substrate**, 100mL

34095 **SuperSignal West Femto Chemiluminescent Substrate**, 100mL

General References

1. Kaufmann, S.H., *et al.* (1987). The erasable Western blot. *Anal Biochem* **161:** 89-95.
2. Kaufmann, S.H. and Kellner, U. (1998). Erasure of Western blots after autoradiographic or chemiluminescent detection. In *Immunochemical Protocols*. Ed. Pound, J.D. Humanna Press, Totowa, NJ., p. 223-35.