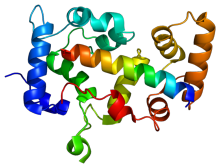
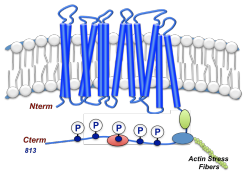


Protocol for Blot Reprobing / Stripping



Introduction
Nitrocellulose and PVDF membranes that have been probed by Western blotting procedures and detected by chemiluminescent or other nonprecipitating substrates may be stripped and re-probed using Western Blot Stripping Buffer. One advantage of chemiluminescence is the possibility to strip and reprobe the protein mixture on the membrane.

Procedure for Stripping an Immunoblot using Pierce Restore Stripping Buffer
21059 Restore Western Blot Stripping Buffer, 500 ml

Notes: Blots that cannot be stripped immediately after chemiluminescent detection may be stored in phosphate buffered saline (PBS) at 4°C until the stripping procedure can be performed.

1. Warm bottle of Restore™ Western Blot Stripping Buffer to room temperature.

2. Place the blot to be stripped in Restore 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 10 ml).
Note: *Optimization of both incubation time and temperature is essential for best results. In general, higher affinity antibodies will require at least 15 minutes of stripping and may require an incubation temperature of 37°C.*

3. Remove the blot from the Restore Western Blot Stripping Buffer and wash in Wash Buffer.

Optional: Test for complete removal of the HRP label (e.g., secondary antibody): Incubate the membrane with new chemiluminescent detection reagent and expose to film. If no signal is detected using a 5-minute exposure, the HRP conjugate has been successfully removed from the antigen or primary antibody.

If signal is detected return to step 2, stripping for an additional 5-15 minutes. 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.

Notes: Blot may be stripped and reprobed several times but may require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobings may result in decreased signal if the antigen is labile in Stripping Buffer. Analysis of the individual system is required.
- Reblocking a membrane is usually not necessary after stripping but may be required in some applications.

Protocol for "homemade" stripping buffer
1. After film exposure, wash membrane four times for 5 minutes each in TBS/T. Best results are obtained if the membrane is not allowed to dry.
2. Incubate membrane for 30 minutes at 50°C in stripping buffer (with slight agitation).
3. Wash membrane six times for 5 minutes each in TBS/T.

References
1. Kaufmann, S.H., Ewing, C.M. and Shaper, J.H. (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*.