

Western blot membrane stripping for restaining protocol

Stripping is the term used to describe the removal of primary and secondary antibodies from a western blot membrane. Stripping is useful when one wants to investigate more than one protein on the same blot, for instance a protein of interest and a loading control. When probing for multiple targets, stripping and re-probing a single membrane instead of running and blotting multiple gels has the advantage of saving samples, materials, and time.

It is not advisable to make quantitative comparisons of targets probed before and after stripping since the procedure removes some sample protein from the membrane. For the same reason, a stripped membrane should not be probed to demonstrate the absence of a protein.

A PVDF membrane is highly recommended to minimize loss of sample protein. Note also that colorimetric/chromogenic detection reagents will leave a permanent visible stain on the membrane that can interfere with subsequent detection of targets of similar molecular weights. Chemiluminescent reagents such as ECL are recommended as they will not leave a stain and are more sensitive than colorimetric reagents.

The following two protocols differ in harshness of treatment. As a rule of thumb, try the gentler one first and then proceed to the harsher one if there is still a signal from the antibody that one is trying to strip. These steps can be repeated for probing with several antibodies, though the potential signal may be weaker and the background higher after each round of stripping. Some researchers report successfully staining a membrane after stripping ten or more times.

Efficiency of stripping can be checked by incubating the membrane with chemiluminescent detection reagent. If stripping is judged to be satisfactory, rinse the membrane several times with buffer, then block before proceeding to the antibody incubation.

Mild stripping

Buffer, 1 L

15 g glycine
1 g SDS
10 mL Tween 20
Dissolve in 800 mL distilled water
Adjust pH to 2.2
Bring volume up to 1 L with distilled water

Procedure

1. Use a volume that will cover the membrane and incubate at room temperature for 5–10 min
2. Discard buffer
3. Repeat incubation for 5–10 min with fresh stripping buffer
4. Discard buffer
5. Wash for 10 min in PBS
6. Wash for 10 min in PBS
7. Wash for 5 min in TBST
8. Wash for 5 min in TBST
9. The membrane is now ready for blocking

Harsh stripping

Prepare buffer and strip membranes under a fume hood.

Buffer, 0.1 L

20 mL SDS 10%

12.5 mL Tris HCl, pH 6.8, 0.5 M

67.5 mL distilled water

Add 0.8 mL β -mercaptoethanol under the fume hood

Procedure

1. Warm the buffer to 50°C
2. Add the buffer to a small plastic box which has a tight lid. Use a volume that will cover the membrane
3. Add the membrane. Incubate at 50°C for up to 45 min with some agitation
4. Dispose of the solution as required for β -mercaptoethanol based buffers
5. Rinse the membrane under running water tap for 1–2 min
6. Traces of β -mercaptoethanol will damage the antibodies; wash extensively for 5 min in TBST
7. The membrane is now ready for blocking