Western blot stripping

1. Background and purpose of the procedure

It is possible to remove the antibodies bound to a nitrocellulose or PVDF Western blot (immunoblot) membrane without affecting bound epitopes. This procedure allows for removal of bound antibodies and re-probing membrane using same specie antibodies but for a different epitope. When blots have been revealed with chromogenic substrate such as DAB, bound antibodies can be removed by stripping but chromogenic bands on membrane will stay intact.

2. Materials and equipment

- Blot membrane (nitrocellulose or PVDF)
- Tris-buffered saline wash solution, TBS-T (*see 3. Recipes*)
- Stripping buffer (acidic or SDS) (*see 3. Recipes*)
- ddH_2O

- Bench rocker
- Incubation tray with lid
- Hybridization oven at 60˚C (for SDS stripping)

3. Recipes

a. Tris-buffered saline (TBS) 10X

250 mM Tris-base (Fisher Bioreagents, Molecular Biology, BP152-500) *[24.2 g for 1 L]*
1.37 M Sodium chloride (Fisher Chemical, Certified ACS, S271-1) *[80 g for 1 L]*
ddH_2O qsp 1 L

Adjust pH to 7.6 using HCl (Fisher Chemical, Certified ACS Plus, A144-500)

b. Tris-buffered saline wash solution (TBS-T)

1/10 TBS 10X *[100 mL for 1 L]*
0.1% Tween 20 (Fisher Bioreagents, BP337-100) *[1 mL for 1 L]*
ddH_2O qsp 1 L

This solution can be stored for up to 6 weeks at room temperature.

c. Acidic stripping buffer

0.1 M Glycine (Fisher Bioreagents, Crystalline powder, BP381-1) *[751 mg for 100 mL]*
0.5% Tween 20 (Fisher Bioreagents, BP337-100) *[500 µL for 100 mL]*
0.1 M β-mercaptoethanol (Fisher Bioreagents, Electrophoresis, BP176-100) *[700 µL for 100 mL]*
ddH_2O qsp 100 mL

Adjust pH to 2.1. This solution can be stored for 6 months at 4˚C without β-mercaptoethanol. β-mercaptoethanol can be added just before use.

d. SDS stripping buffer

0.1 M Tris-HCl (Fisher Chemical, Certified ACS, T395-500) *[1.21 g for 100 mL]*
2% Sodium dodecyl sulfate (Fisher Bioreagents, Electrophoresis, BP166-500) *[2 g for 100 mL]*
0.1 M β-mercaptoethanol (Fisher Bioreagents, Electrophoresis, BP176-100) *[700 µL for 100 mL]*
ddH_2O qsp 100 mL

Adjust pH to 6.8. This solution can be stored for 6 months at room temperature without β-mercaptoethanol. β-mercaptoethanol can be added just before use. SDS may precipitate, heating the solution at 60˚C will allow SDS back in solution.

4. Method

a. Rinse the membrane twice with ddH_2O
b. Add stripping buffer to the membrane and incubate at room temperature under constant agitation on rocker for 30 min *[for acidic stripping buffer]*
c. Add stripping buffer to the membrane and incubate at 60˚C in hybridization oven for 30 min *[for SDS stripping buffer]*
d. Transfer membrane in a clean incubation tray
e. Rinse the membrane with three large ddH_2O washes to remove any traces of stripping buffer
f. Rinse the membrane with five TBS-T washes of 5 min on rocker
g. Membrane can be used for re-probing using standard Western blotting (immunoblotting) protocol

5. References

Litovchick L. Stripping of the Immunoblot for Reprobing. Cold Spring Harb Protoc. 2020 Mar 2;2020(3):098491. doi: 10.1101/pdb.prot098491. PMID: 32123013.

6. Revision history

Revision #	Date	Prepared by
1.0	**2021-09-25**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		