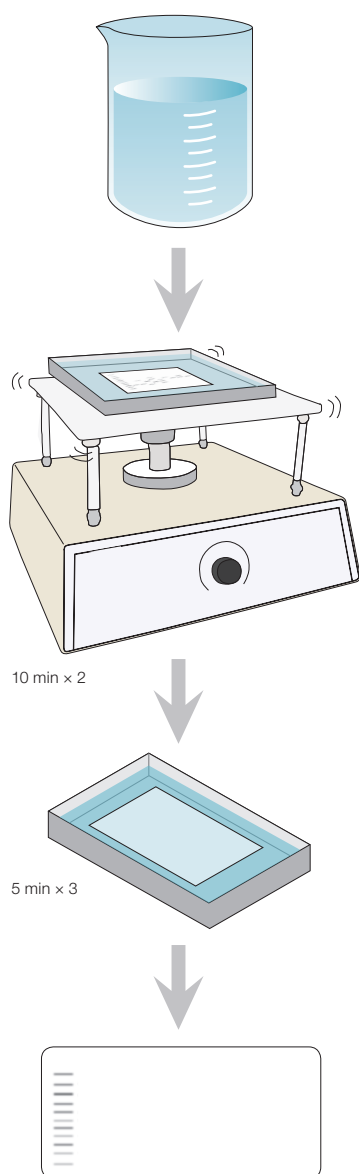


Blot Stripping and Reprobing

Protocol

Bulletin 6218

Based on Legocki and Verma 1981



1 Prepare acidic glycine stripping buffer (0.1 M glycine, 20 mM magnesium acetate, 50 mM KCl, pH 2.2; for recipe.

2 Add enough acidic glycine stripping buffer to completely cover the developed membrane and incubate at room temperature for 10 min with gentle agitation.

3 Repeat step 2 with fresh acidic glycine stripping buffer.

4 Wash the blot three times in TTBS for 5 min each with gentle agitation.

5 Test for complete removal of primary antibody by reprobing with only the secondary antibody and redeveloping. No signal should be detectable. Re-block the membrane and proceed to the next detection protocol.

TIPS

This protocol (based on Legocki and Verma 1981) uses low pH to gently remove antibody from the membrane. The protocol removes little of the sample proteins but may not remove all antibodies with high affinities for their targets

BIO-RAD

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).



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