Enhanced chemiluminescence has been demonstrated to be a sensitive, rapid method of detecting horseradish peroxidase labelled proteins. The following method Kaufmann et al. (1) for stripping Western blots has been used successfully with ECL™ Western Blotting Detection Reagents. Stripping and reprobing blots saves time and sample by enabling researchers to re-use a single blot with different primary antibodies.

### Methods

1. Prepare Western blots by separating rat kidney lysate on polyacrylamide gels at constant voltage and transferring onto Hybond™ ECL overnight at 30 V.

2. Block membrane with 10% non-fat dried milk in TBS containing 0.1% Tween™ 20 (TBS-T) for 1 h.

3. Wash membrane 3 x 5 min in TBS-T.

4. Incubate blot with the primary antibody monoclonal anti-actin 1:3000 in TBS for 1 h.

5. Repeat wash Step 3.


7. Wash membrane 3 x 5 min in TBS 0.3% Tween 20.

8. Repeat wash Step 3.

9. Incubate membrane for 1 min with ECL Western Blotting Detection Reagents (0.125 ml/cm²); drain reagents and wrap membrane in plastic wrap before exposure to Hyperfilm™ ECL for 15 s.

10. Wash membrane 2 x 10 min in TBS-T.

11. Strip membrane with 2% SDS, 100 mM mercaptoethanol in 62.5 mM Tris-HCL pH 6.8 for 30 min at 70 °C. Lower temperatures (50–70 °C) may be adequate for stripping, however this must be determined empirically with individual antibodies.

12. Repeat wash Step 10.

13. Incubate membrane with ECL Western Blotting Detection Reagents, drain, wrap in plastic wrap and expose to Hyperfilm ECL for 1 min to check for signal removal (see Fig 2).

14. Cut membrane to:
   a) detect a different antigen, β-tubulin (membrane I)
   b) relabel actin (membrane II)

   Block both sections as in Steps 2 and 3.

15. Incubate membrane I with 1:2000 mouse monoclonal anti-β tubulin in TBS. Incubate membrane II again in mouse monoclonal anti-actin at 1:3000 in TBS. Incubate both for 1 h.

16. Wash membranes as in Step 3.


18. Wash membranes as in Steps 7 and 3.

19. Incubate membranes with ECL Western Blotting Detection Reagents, drain, wrap in plastic wrap and expose to Hyperfilm ECL for 15 s.
Results

Fig 1.
Rat kidney lysate Western blot detected with mouse monoclonal anti-actin and HRP-conjugated anti-mouse IgG 15 s exposure to Hyperfilm ECL.

Fig 2.
Stripped blot from Figure 1. Blot was detected again with ECL to confirm removal of antibodies. 1 min exposure to Hyperfilm ECL.

Fig 3.
Membrane I was detected after stripping with mouse monoclonal anti-β tubulin and HRP-conjugated anti-mouse IgG.
Membrane II was redetected for actin as in Figure 1.
Membrane III shows labelling with actin and β-tubulin for identification purposes. All 15 s exposures to Hyperfilm ECL.

Discussion

Figure 3 shows that following membrane stripping it is possible to re-detect the same antigen or detect a different antigen. Thus suggesting that a Western blot can be stripped, without damage to antibody labelled antigens or unlabelled antigens, and subsequently detected with ECL Western Blotting Detection Reagents.

Following the second detection and stripping procedures, blots were re-incubated with secondary HRP-conjugated antibody to check for the presence of any remaining primary antibody. No signal was detected (result not shown).

It should be noted that when stripping primary and secondary antibodies some of the membrane bound antigen may also be removed resulting in reduced sensitivity in subsequent detections.

Warning

Safety procedures as set out in the product literature should be observed when using the products described here.

Products used

Amersham Biosciences products used for this process:
Hybond ECL (20 × 20 cm) RPN2020D
ECL Western Blotting Detection Reagents RPN2106
Hyperfilm ECL (18 × 24 cm) RPN2103K

Other materials required

Twee 20

References


Product information

for further details:

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