~~100 ml strip soln~~

100 mM 2ME 0.7ml
2% SDS 20ml 10
62.5 mM Tris Hcl pH 6.7 3.125ml 2M

100ml strip soln

Stripping and reprobing pr

From Amersham's West blotting team

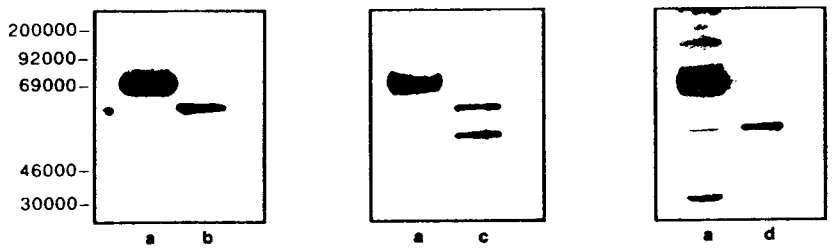

Figure 2. Reprobing Western blots. C6 rat glial cell lysate separated by 12% SDS/PAGE and transferred to Hybond-ECL. Rainbow marker sizes are given.

a) Rainbow markers (RPN 756). Note that various 'rainbow coloured' marker proteins 'light up' differently according to different cross-reactivity with the primary antibody.

b) Detection of tubulin using mouse monoclonal anti β-tubulin (N 357) (1:1000) and anti-mouse Ig horseradish peroxidase linked whole antibody (from sheep) (NA 931) (1:2500).

c) Same blot reprobed with mouse monoclonal anti-actin (N 350) (1:1000) and anti-mouse Ig horseradish peroxidase linked whole antibody (from sheep) (NA 931) (1:2500).

d) Detection of actin using mouse monoclonal anti-actin (N 350) (1:1000) and anti-mouse Ig horseradish peroxidase linked whole antibody (from sheep) (NA 931) (1:2500).

Blots detected by ECL and exposed to Hyperfilm-ECL for 15 seconds.

Protocol 1

Reprobing blots with a second primary antibody

Sequential reprobing of membranes with a variety of antibodies is possible with the ECL Western blotting system (RPN 2106), following the steps below. The membranes may be stored wet wrapped in SaranWrap at 4°C after each immunodetection.

1. Wash membrane for 2×10 minutes in tris-buffered saline-Tween-20 (TBS-T), or phosphate-buffered saline-Tween-20 (PBS-T), at room temperature on a roller incubator, using large volumes of washing buffer. See protocol booklet for full details.

2. Block the membrane by immersing in 5% dried milk in TBS-T or PBS-T for 1 hour at room temperature.

3. Perform immunodetection as described in the protocol booklet for the ECL Western blotting detection system.

Protocol 2

Stripping and reprobing blots

The complete removal of primary and secondary antibodies from membranes is possible following the method outlined below. The membranes may be stripped of bound antibodies and reprobed several times. Blots should be stored wet wrapped in SaranWrap at 4°C after each immunodetection.

1. Submerge the membrane in stripping buffer (100mM 2-mercaptoethanol, 2% sodium dodecyl sulphate, 62.5mM tris-HCl pH 6.7) and incubate at 50°C for 30 minutes with occasional agitation.

2. Wash the membrane for 2×10 minutes in TBS-T or PBS-T, at room temperature using as large a volume of buffer as possible.

3. Block the membrane by immersing in 5% dried milk in TBS-T or PBS-T for 1 hour at room temperature.

4. Perform immunodetection as described in the protocol booklet for the ECL Western blotting detection system.

The ability to reprobe Western blots several times is particularly valuable in a number of situations:

- When only small or valuable samples are available
- When unexpected results need to be confirmed by probing with a second antibody
- When a purification protocol is evaluated for the presence of more than one antibody

Working solutions for ECL stripping and reprobing

Tris buffered saline (TBS) pH 7.6:
2.42g tris base (20mM)
8g sodium chloride (137mM)
Adjust pH to 7.6 with 1M hydrochloric acid
Dilute to 1000ml with distilled water and check final pH

Phosphate buffered saline (PBS) pH 7.5:
11.5g disodium hydrogen orthophosphate anhydrous (80mM)
2.96g sodium dihydrogen orthophosphate (20mM)
5.84g sodium chloride (100mM)
Dilute to 1000ml with distilled water and check pH

PBS-Tween (PBS-T) and TBS-Tween (TBS-T):
A 0.1% Tween-20 concentration in PBS or TBS is suitable for most ECL Western blotting work on nitrocellulose, but concentrations varying from 0.05% to 1% may be required to suit your specific experiment.

For additional details, please request TechTip 122 from your Amersham representative or circle reader reply card number 12.