Western Blot and Ponceau-S Staining

Western Blot

	Buffer T3	Buffer T2	Buffer T1 (fresh)
Water	500 ml	775 ml	100 ml Buffer T2
1 M Tris-Hcl pH 10.4	300 ml	25 ml	525 mg 6-Aminocapronnic acid
Isopropanol	200 ml	200 ml	

Work with gloves!

1. Cut 10 Whatman papers and one Nitrocellulose membrane to 9 x 6 cm (this stuff is expensive, avoid wasting; never touch the membrane without gloves!); label membrane with a pencil at the upper right corner, this will become the side to which proteins are blotted.
2. In one corner of a large glass plate, soak 3 Whatman papers with buffer T3 and squeeze out bubbles by rolling over the stack with a test tube; add a little more buffer 3 and place the three filter papers onto the anode of a semi-dry blotting device.
3. In another corner of the glass plate, soak 2 Whatman papers with buffer T1 and squeeze out bubbles with a fresh test tube; add a little more buffer T1.
4. Place gel left side right and without bubbles onto the two Whatman papers.
5. Soak Nitrocellulose membrane in buffer T2 and place it onto the gel such that the pencil mark faces the gel.
6. Soak 2 more Whatman papers in buffer T2 and place them onto the membrane; squeeze out bubbles with a test tube starting from the middle into all directions (don't apply too much pressure!).
7. Take the entire stack, turn it upside-down and place it onto the three Whatman papers soaked with buffer T3 that are already on the anode.
8. In another corner of the glass plate, soak 3 Whatman papers with buffer T1 and squeeze out bubbles with a fresh test tube; then place the three papers onto the sandwich on the anode.
9. Close blotting device by placing the cathode on top and conduct transfer for 60 min by applying a current of 0.8 mA/cm2 per gel surface.

Ponceau-S Staining

Work with gloves!

1. Disassemble the sandwich after the transfer and incubate membrane for 1 min in Ponceau–S solution (touch membrane only with forceps! Be careful, it is very fragile!)
2. Pour the Ponceau solution back into its container (it is re-used several times) and wash membrane with distilled water until bands appear brightly without background.
3. Take an image of the membrane using the FUSION device.