Western Blot

Western Blot protocol

For Purified Protein:

- Prepare 2, 5, 10 and 20 ul of protein (in elution buffer with DoDM) adding Loading Sample Dye 2x with 5% BME.
- Boil the samples for 5 minutes, spin them down and load them on the gel with the long tips.

*Note: this protocol is good for any denaturing gel, either for westerns or gel staining.*

- Let the gel run at the beginning with constant Volts at 80 and then after the forming of the line of the front gel, go up to 130 volts. Don’t go above 150 Volts!
- When the gel front reaches the bottom of the gel, detach the two plastic walls and recover the gel. Cut the very bottom out.

- For gel staining using Sypro Ruby (S12000 1 L Invitrogen), put the gel in a box and fix it with this ratio of reagents: 50 Methanol: 10 Acetic Acid: 40 double distilled water.
- After fixation, add enough Sypro Ruby solution to cover the gel. Let over night. The day after, wash the solution with the fixing solution. Do at least two washes and and see the gel under UV light.

For silver staining, look at the manual product LC6070 from Invitrogen.

- For Western Blot transfer the gel to a PVDF membrane. If you don’t use the iBlot, remember to activate the membrane by wetting it in methanol for few seconds.
- If you use the iBlot, use the program for 7 minutes and follow the manual. If you use the completely wet transfer system, wet the sponges and the 3mm filter in the transfer buffer with methanol 20%.

With this system transfer fro 1 hour at 100 volts in cold buffer.

- After transfer, with whatever method, let the filters dry by air. Wet them back in methanol and put them in blocking solution: PBST + 5% milk.

*Note: if you are dealing with a blue native gel, before the blocking wash the filter in methanol until the blue disappears or becomes very light!*

- After 1 hour add the primary antibody.
- Wash the filter 3 times with PBST for 5 minutes and add secondary antibody (usually 1:10,000 diluted) in blocking solution. Let for at least 1 hour, don’t go over 3 hours!
- Wash 3 times like above.
- Add ECL and develop film t use alpha imager.

*Note: see also antibodies manuals for information about western blot or electrophoresis protocols.*