

## **Protein prep from C. elegans and Western Analysis**

Pasquinelli lab protocol (adapted from Reinhart and Ruvkun, 2001)

1. Wash the worms off of plates in M9 and rock the population for 20 min. at RT to make them digest bacteria in the gut. Wash once more with M9 at the end of incubation and transfer the worms to screw cap tubes.
2. Centrifuge the tubes for 30 seconds at 5000g to pellet the worms at the bottom of the screw cap tubes. For eggs, let the tubes stand at RT for 2-3 min before centrifuging at 1000g, eggs tend to pellet at the walls of the tube at higher speed. Note down the packed volume of worms/eggs before freezing in dry ice/ethanol.
3. While the worms/eggs are still frozen, add an equal volume of 2X SDS buffer (Reinhart et al, 2001).

### **2X SDS buffer (5 ml)**

20% SDS (4% final)	1ml
2M Tris.Cl, pH 6.8 (100mM final)	250ul
Glycerol (20% final)	1ml
H2O	2.75ml

4. For larval stages, directly boil the worm pellet in 2X buffer for 15 min vortexing the samples once after 7-8 min. For eggs, homogenize the samples using hand-held homogenizer before boiling. Centrifuge the tubes at 10,000g for 5 min to pellet the insoluble material.
5. Transfer the supernatant to a separate tube and save the pellets till protein estimation.
6. Protein Estimation by Bradford method. The glycerol in the 2x buffer tends to mask the protein quantitation so it is important to dilute the samples and also estimate the standards in the presence of the buffer used for samples. Set up the tubes as follows:

For Blank: 5 ul H2O + 1ul of 1X SDS buffer

For Standards: 5ul of std protein solution (0.25 to 2 ug/ul making the curve 1.25 to 10ug value) + 1ul of 1X SDS buffer

For Samples: 5 ul H2O + 1ul of sample

7. Prepare protein samples to load on gel as follows:

x ul (10ug of protein) + 1X SDS buffer to make total volume 17.8 ul + 2ul of 1M DTT + 0.2 ul of 10% bromophenol blue.

Boil samples for 4 min, spin down at highest speed and load on a 4-20% gel from invitrogen (EC6025BOX). Use 1X running buffer after diluting the 10X.

## **Running the gel**

1. Run the gel at 100V for 1 hr and increase the voltage to 200V for further running; Run till bromophenol blue reaches the bottom.
2. Open the gel cast; For staining the gel put it in staining solution (Invitrogen) for 1 hr on a shaker. For destaining put the gel in H2O for 30 minutes, change H2O if further destaining is required. Do not stain the gel if you want to transfer it.

## **II Setting up the transfer**

- Transfer buffer (2l): Tris base- 6g; glycine- 29g; methanol- 400ml; make up volume with water. Always make fresh transfer buffer.

- Cut the PVDF membrane and four whatmann sheets to the same size as the gel

1. PVDF membrane treatment: Rinse the membrane in methanol for 15 seconds, 2 minutes in water and 5 minutes in transfer buffer. Do not let the membrane dry and keep in transfer buffer while setting up everything else.
2. Rinse the gel once in the transfer buffer
3. For transferring proteins upto 120kDa, do at 250 mAmps for 4 hours in the cold room. Proteins greater than 120kDa, add 0.05% SDS to the transfer buffer and allow transfer to go on for atleast 6 hours.

### **III Developing the membrane**

- 1XPBST: 1X PBS, 0.1% Tween 20
- Blocking buffer: 1X PBST, 5% non fat milk powder
- Washing buffer: 1XPBST

All the steps are carried out with shaking at RT or 4 °C

1. After the transfer put the blot in blocking buffer for 1 hour at RT or 4 °C O/N.
2. Incubate with primary antibody diluted in blocking buffer for 1 hour at RT or 4 °C O/N (depending on the antibody).
3. Wash the blot 4 times for 10 min each at RT with washing buffer
4. Incubate with secondary antibody diluted in blocking buffer for 1 hr at RT
5. Wash 3 times for 10 min each at RT with washing buffer
6. Treat the membrane with developing reagent. Use ECLplus (GE) reagent following manufacturer's instructions (Reagent A:B=1:40). Do not let the membrane dry and wrap in a saran wrap for exposing on film. Take 1', 2', 5', 10' exposures.