Western blot protocol

Sample preparation

1. Add loading buffer (NuPage LDS sample buffer) to the samples at a ratio of 1:3.
2. Spin down the samples (quick spin).
3. Incubate the samples at 70°C for 10 minutes.

Gel and electrophoresis apparatus preparation

1. Wash all the parts of Cell SuperBlock apparatus with water.
2. Combine everything together (only one way to do it).
3. The ready for use gel (Invitrogen) is in the transparent fridge in the back of the bottom shelf.
4. Take off the sticker and the comb and wash the gel with water.
5. Put the gel in the apparatus with the wells turning to the inside. If you need only one gel, put a plastic barrier where the other gel should be.
6. Add Running buffer (NuPAGE SDS Running Buffer), first between the gels, and after you check for leaks, add buffer outside the gels as well. The buffer should cover the wells on the inside, and on the outside about half the height of the gel. (to make new running buffer see recipe at the end of the protocol).

Loading samples to the gel

1. Spin down the samples again for few seconds.
2. Loading is done with special tips from the "western" drawer.
3. Load 8-10µL of each sample to the gel (you can load up to 25µL to each well).
4. After you finished loading all the samples to the gel, "quick spin" the marker (PageRuler – from the big freezer), and load 5-8µL to the gel.
5. Connect the electrodes to the power source and run the gel in 180V for an approximately 1 hour.

Transfer "sandwich"

1. Cut a membrane in the same size of the sponge (both in western drawer).
2. Put the membrane in methanol bath (incubate for 3 minutes).
3. Prepare a bath with cold transfer buffer (to make new transfer buffer see recipe at the end of the protocol).
4. Remove the gel out of the plastic casing, cut the protruding bottom part of the gel and put the gel in the buffer.
5. Wash the membrane 3 times with water – make sure that the membrane doesn't dry out.
6. Make the sandwich: The black part of the plastic should be on the bottom:
   UP
   - Transparent plastic
   - sponge
   - gel
   - membrane
   - sponge
   - black plastic

   DOWN

7. Roll a test tube over the sandwich to take out any bubble.
8. Close the sandwich and insert it to the transfer apparatus with the black side of the sandwich facing the red side of the apparatus.
9. Add icepack to keep the temperature of the buffer and add transfer buffer until it covers the sandwich.
10. Connect the electrodes to the power source and run it at constant current at 180A for 1 hour.
11. After 1 hour take the membrane out of the sandwich and mark the Ladder right side and the wells locations with normal pen.
12. Prepare blocking solution: 10% skim milk in TBST buffer (1g in 10 ml buffer). Put the membrane in the buffer for 20 min (room temperature)->overnight (at +4 ^{o}C).
13. Primary antibody incubation: prepare primary antibody solution: 5% skim milk in TBST buffer and add (rabbit) anti-E.coli adenylate kinase IgG 1:5000. Put the membrane in the solution for 1hour.
14. Wash the membrane 3 times with TBST for 10 minutes.
15. Secondary antibody incubation: prepare secondary antibody solution: 5% skim milk in TBST buffer and add HRP-conjugated (mouse)-anti-(rabbit) polyclonal IgG 1:5000. Put the membrane in the solution for 1hour.
16. Wash the membrane 3 times with TBST for 10 minutes.

Developing process

The whole developing process is done in the dark because luminol is sensitive to light!

1. Prepare developing solution: 1:1 hydrogen peroxide (H_2O_2) and Luminol (1.5 ml each).
2. Cover the tube with aluminum foil.
3. Wash the plastic cover and a small bath with water and soap.
4. Remove the membrane from TBST, remove the excess of liquid by gentle pressing the membrane to paper.
5. Put the membrane in the bath and cover facial side with developing solution and shake for 5 minutes.
6. Remove the excess of liquid. Put the membrane in the plastic cover so it won't dry out.
7. Photograph the membrane with LAS-4000 in chemluminescence mode.

Running buffer 1L:

- 50ml NuPAGE SDS running buffer X20 stock.
- 950ml DDW.

Transfer buffer 1L:

- 100ml TGSX10 stock.
- 200ml metOH.
- 700ml DDW.
- Keep at 4°C.