Western blotting

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Abstract

Basic Western blotting with TURBO transfer

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Protocol

Blottin

Step 1.
Run your protein samples on SDS-PAGE as usual

Step 2.
Soak 6 pieces of Whatman blotting paper, nitrocellulose membrane (all a bit bigger than your gel) and your SDS-PAGE gel in transfer buffer for a few minutes

Step 3.
Assemble the blot: 3 layers of Whatman paper, nitrocellulose membrane (to the positive pole of the device; in the Bio-Rad blotting machine to the bottom), SDS-PAGE gel, 3 layers of Whatman paper

Step 4.
Roll out all possible air bubbles with a tube or some other device

Step 5.
Start blotting: with the Bio-Rad machine either 30 min programme, or TURBO (TURBO might not be always the best option as not all proteins will transfer)

Antibody treatment

Step 6.

Blocking: Put nitrocellulose membrane in to box and add blocking solution, let it incubate 1,5 h, RT, at shaking

Step 7.

Primary antibody: Add primary antibody solution, incubation recommended O/N, shaking, +4 °C. Can also be done at RT for 1-2 h, but less specific binding and some antibodies might degrade at RT. Collect primary antibody solution, it can be used about 10 times, add 1 % NaN3 and store in +4 °C.

Step 8.
Washing: Rinse twice with PBS-T, and wash with PBS-T 5 min at shaking three times

Step 9.
Secondary antibody: Add secondary antibody solution, incubate 1 h, RT, shaking

Step 10.
Washing as earlier

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Step 11.
Mix chemiluminesence detection solutions 1 + 1 mL

Step 12.
Place the membrane on a plastic sheet and pipet the detection solution on the membrane

Step 13.
Fold the plastic and let the solution react at least 1 min

Step 14.
Within the next hour, image with Fujifilm LAS 3000 imager. A 10 sec exposure x 3 should give good images. Remember to take a digitized version without moving the membrane, so you can easily have the molecular weight standard in your image. Exposure time here should be 1/60 sec or 1/100 sec.