WESTERN BLOT

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

PROTOCOL:

1. Sample preparation and lysis

- Take 750 µl of fresh liquid culture and centrifuge them 12 000 RPM x 2 min
- Remove the supernatant
- Freeze the pellet
- Resuspend the pellet in 50 µl of 2X Laemmli buffer Heat the sample to 95 °C for 5 min.
- Centrifuge for 10 min at 14 000 RPM Collect the supernatant.
- Place a polyacrylamide gel into a running tank, fill with running TGS buffer. Load 5 ul of prestained MW markers in the first well. Load 5µl of extract in each well.
- Migrate for 50 min at 150 volts

2. Transfer to membrane

- Carefully lay the polyacrylamide gel onto a nitrocellulose membrane (TransBlot Turbo Transfer pack, Bio-Rad) inside the TransBlot Turbo cassette. Remove bubbles.
- Run transfer, adjusting the duration to the gel thickness and the size of the proteins of interest..
- Once the program is over, check that the prestained markers have homogeneously transferred to the membrane. Place the membrane in a container, rinse with water.

3. Western Blot fixation and revelation

- Block the membrane with 10 ml of TBS tween 0.05% + 5% milk for 10 minutes under gentle agitation.
- Incubate with the primary antibody diluted in TBS tween 0.05% + 5% milk (for instance anti-his antibody diluted 1/1000). Incubate overnight at 4°C on a shaker.
- The next day rinse quickly 3 times with TBS, then wash 2 more times for 5 min under agitation.
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- Incubate with secondary antibody conjugated to HRP for 1 hour on a shaker (for instance anti-mouse antibody conjugate HRP diluted 1/10000).
- Rinse quickly 3 times with TBS, then wash 2 more times for 5 min under agitation.
- Prepare the ECL reagent (ECL Plus, Pierce): mix 2.5mL of each substrate.
- Incubate the membrane with the mix for about 30 seconds. Soak excedent liquid, place the membrane on the tray of the ECL reader.
- Image the membrane with the CCD camera (Amersham Imager)