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Western Blot

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ABSTRACT

This is a protocol of Leo Parra-Rivas, PhD.

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Protocol status: Working
We use this protocol and it's working

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Preparing Proteins

1 Dilute protein samples to [M] 1.25 µg/µL. For running a 10-well gel, each loading sample should be around 15 µL to 20 µL.

2 Add 4x sample buffer to protein samples.

3 Denature protein at 95-100 °C for 00:05:00 to 00:10:00 15m

Running the Gel

4 Secure the gel (NuPAGE 4-12% Bis-Tris Gel; 1.5mm 10 wells) into the module. Fill the tank with run 1h 15m buffer. Load 15 µL of the ladder, and 20 µL of the samples (25ug/20ul, more if less concentrated).
Run at 70-80V for 01:15:00. Until the blue band reaches the end of the gel, run for 10 more min.

4.1 Ensure the strip on the bottom is removed. Remove the comb without breaking the wells.

4.2 Running Buffer: 1900ml H2O, 100ml Running buffer 20X

4.3 You may add more buffer if the buffer level is low.
You may increase the voltage to 100V.

Transferring the Gel

5 Assemble the module according to instructions (membrane = 0.2ul pores; gel needs to be flipped) 1h 25m
Ensure all bubbles are removed.
Add transfer buffer (ice cold) to the tank. Securely locate the module. Add ice around the tank before running.

Run at 25-30V for 01:25:00

5.1 transfer buffer: 1500ml H2O, 400ml methanol, 100ml transfer buffer 20X

Staining

6 Fixation: rinse membrane with 1xPBS. Incubate in 0.4% PFA for 00:30:00. 30m

7 Blocking: wash with 1xPBS, 3 times 00:10:00. Block with 5-10% milk for 01:00:00. 1h 10m

8 Primary antibody: Specific primary antibody diluted in 5% nonfat dried milk in TBST, 4 °C overnig 30m with rocker.
Wash: Recover the primary antibody, and rinse with wash buffer. Wash for 00:30:00 with wash buffer

8.1 Wash buffer: 1800ml H2O, 200ml 10X PBS, 2ml Tween-20

9 Secondary antibody: Secondary antibody 1:2000 in milk, 01:00:00 Room temperature. Add 1h approximately 10 ml to each tray.
Wash: Rinse and wash with wash buffer. The membrane can be stored in the wash buffer

Imaging 5m

10 Mix imaging reagent 1:1. Either apply reagent to membrane before imaging, or incubate the membran 5m mixed reagent for 00:05:00 while rocking.
Avoid bubbles when placing the membrane on the machine.
Single image, start with 0.1s exposure and gradually increase until desired exposure is achieved.
More reagent can be added if signal is reduced.