Western blotting using the BioRad Criterion Blotter system

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

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ABSTRACT

Protocol for performing an SDS-PAGE Western blot analysis using the BioRad Criterion Blotter system.

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Day 1

1h 47m

1 Load samples onto a 4-12% Bis-Tris (NuPage) gel with MOPS buffer (ThermoFisher). If using SDS loading dye (with 5% SDS final concentration), all lanes must contain the same volume of sample. Ensure all lanes are filled with 1x SDS loading dye at the same volumes as the sample/marker lanes. If this is not done, samples will spread according to the total sample volume, and the wells will not run evenly.

2 Run gels according to the following gradient: 100 V (00:10:00), 150 V (00:10:00), 200 V (until the dye front has reached the desired distance – typically ~40 min). 20m

3 Cut 1x piece of PVDF (0.45 um), and 2x pieces of Whatman paper per gel, according to the gel dimensions.

4 Place the PVDF membrane into methanol for ~ 5 seconds, remove the methanol and wash the membrane three times in distilled water.

5 Place the membrane in cold (4 °C) 20% methanol Bis-Tris transfer buffer (Thermofisher)

6 Remove the gel from the cassette and place in ice cold 20% methanol Bis-Tris transfer buffer (ThermoFisher)

7 Add a frozen ice pack to the Criterion Blotter transfer tank, and fill the tank with cold transfer buffer.

8 Soaking each Whatman paper piece in transfer buffer for ~ 5 sec before use, assemble the following transfer stack: 1x Whatman, SDS-PAGE gel, PVDF membrane, 1x Whatman, rolling each layer to prevent bubbles accumulating between pieces

9 Place the transfer stack between sponges and secure them in the cassette in the correct orientation, and place the cassette in the transfer tank. If transferring 1 gel, ensure an empty cassette containing only sponges is placed in the second cassette holder in the tank.

10 Run the transfer at 100 V for 01:00:00. 1h

11 Remove the membrane from the transfer stack and place into destain (50% methanol/7% acetic acid) for 00:02:00 rocking at Room temperature. 2m

12 Wash the membrane 3x in 0.05% v/v Tween/PBS for 00:02:00 rocking at Room temperature. 2m

13 Incubate the membrane in 3% w/v milk powder in 0.05% v/v Tween/PBS for 00:20:00 rocking at Room temperature. 20m

14 Wash the membrane 3x in 0.05% v/v Tween/PBS for 00:03:00 rocking at Room temperature. 3m

15 Place the membrane in the desired primary antibodies made up in 3% w/v BSA in 0.05% v/v Tween/PBS and incubate rocking, either overnight at 4 °C or for 01:00:00 at Room temperature. 1h

Day 2

1h 14m

16 Wash the membrane 3x in 0.05% v/v Tween/PBS for 00:03:00 rocking at Room temperature. 3m

17 Place the membrane in the desired secondary antibody in 3% w/v milk powder in 0.05% v/v Tween/PBS for 01:00:00 rocking at Room temperature. 1h

18 Wash the membrane 2x in 0.05% v/v Tween/PBS for 00:03:00 rocking at Room temperature. 3m

19 Wash the membrane once in PBS for 00:03:00 rocking at Room temperature. 3m

20 Incubate the membrane in ECL developing reagent for 00:05:00, prior to developing the blot using a ChemiDoc Imaging System. 5m