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Western Blot

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1 Works for me This protocol is published without a DOI.

LIMR

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ATTACHMENTS

Biorad Protein PAGE bulletin.pdf Western Blot Flow Chart.gif

PROTOCOL CITATION

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ATTACHMENTS

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MATERIALS

NAME	CATALOG #	VENDOR
NuPAGE Antioxidant	NP0005	Thermo Fisher Scientific
12-well NUPAGE 10% Tris-Bis gel	NP0302BOX	Thermo Fisher Scientific
Methanol	A452-4	Fisher Scientific
NuPAGE™ MOPS SDS Running Buffer (20X)	NP0001	Thermo Fisher
NuPAGE™ LDS Sample Buffer (4X)	NP0007	Thermo Fisher
NuPAGE™ Sample Reducing Agent (10X)	NP0009	Thermo Fisher
NuPAGE™ transfer buffer	NP0006	Thermo Fisher Scientific

DISCLAIMER:

Gel Electrophoresis

1 Make loading dye using LDS Sample Buffer (4X) and Reducing agent (10X). *For* **20 µl** *of sample, add* **7.5 µl** *sample buffer and* **3.5 µl** *reducing agent, keeping in mind that each well holds* **30 µl** *total.*

2 Combine sample and loading dye and heat at **95 °C** for **00:05:00** .

3 Prepare precast gel by removing comb and the tape at the bottom of the gel chamber, making sure to remove any excess gel that could block the top of the wells. Construct the gel chamber in the gel tank such that the well openings of the precast are facing inwards toward eachother. Fill the gel chamber with **200 mL** (1X) MOPS running buffer and **500 µl** antioxidant.

4 Remove samples from heat and cool **On ice** for **00:05:00** .

5 Spin samples at 10K for **00:05:00**

6 Load samples into gel using hamilton pipette tips. *Be sure to record the order of the samples in the gel noting that samples on the edges of the gel are more likely to become distorted. Placing the marker in the middle most gel will provide a more reliable size ladder for size estimation.*

7 Run gel at 100V for **01:30:00** or until the protein has migrated far enough into the gel.

Transfer

8 While the gel is running prepare **1 L Transfer Buffer** using **850 mL DiH20** , **50 mL 10x Transfer Buffer** , and **100 mL methanol** and place in cold room for duration of gel run.

9 Soak membrane (for PVDF soak membrane in methanol, for nitrocellulose soak membrane in transfer buffer.) *Do not touch membrane with ungloved hands.*
Soak sandwhich components (2 sheets of filter paper and 2 pads) until completely damp. Make sandwhich being careful to avoid trapping any air between layers. Place cassette in transfer tank with transfer buffer and run for 1 hour.

Blocking

10 Remove membrane from sandwhich, noting the orientation relative to the gel. Wash the membrane in 1X PBST for **00:05:00**

11 Block membrane for **01:00:00** with 5% milk in PBST

Blot with desired Ab.

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