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# Western Blot

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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
<a href="#">NuPAGE Antioxidant</a>	NP0005	<a href="#">Thermo Fisher Scientific</a>
<a href="#">12-well NUPAGE 10% Tris-Bis gel</a>	NP0302BOX	<a href="#">Thermo Fisher Scientific</a>
<a href="#">Methanol</a>	A452-4	<a href="#">Fisher Scientific</a>
<a href="#">NuPAGE<sup>®</sup> MOPS SDS Running Buffer (20X)</a>	NP0001	<a href="#">Thermo Fisher</a>
<a href="#">NuPAGE<sup>®</sup> LDS Sample Buffer (4X)</a>	NP0007	<a href="#">Thermo Fisher</a>
<a href="#">NuPAGE<sup>®</sup> Sample Reducing Agent (10X)</a>	NP0009	<a href="#">Thermo Fisher</a>
<a href="#">NuPAGE<sup>™</sup> transfer buffer</a>	NP0006	<a href="#">Thermo Fisher Scientific</a>

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## 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 each other. 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 DiH2O**, **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 sandwich components (2 sheets of filter paper and 2 pads) until completely damp. Make sandwich 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 sandwich, 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.

