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# Biochemical Measures of Neuropathy - Western Blot Stripping

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### Eva Feldman<sup>1</sup>

<sup>1</sup>University of Michigan - Ann Arbor

Diabetic Complications Consortium Tech. support email: rmcindoe@augusta.edu



Lili Liang

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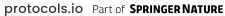


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We use this protocol and it's working

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#### Abstract

### Summary:

Oxidative stress is highly correlated with the metabolic changes caused by hyperglycemia. Increased levels of glucose overload mitochondria and result in the production of reactive oxygen species (ROS). In addition, the flow of excess glucose through cellular pathways decreases the cell's normal ability to detoxify ROS. As a result, the neurons and axons of the peripheral nervous system contain increased levels of ROS and decreased antioxidant capacity. The following assays are used to measure these changes in rodent models of diabetic neuropathy.

#### **Diabetic Complication:**



Neuropathy



### **Materials**

### **MATERIALS**

- **☒** 10% SDS **Gibco Thermo Fisher Scientific Catalog** #15525-025
- **⋈** 0.15 g Dithiothreitol
- 2 mL 0.5 M Tris pH 6.8 Gibco Thermo Fisher Scientific Catalog #15504-012
- **⋈** 6 mL ddH2O

# **Troubleshooting**



## **Performing assay:**

- 1 Rainbow markers do not withstand stripping, so if you don't have a biotinylated marker on your blot, be sure to mark the location of the rainbow markers with a pen or pencil before stripping
  - 1. Incubate your blot for 10-20 min at  $70^{\circ}$ C. to strip. (10 min. for 20-40  $\mu$ g protein and 15-20 min. for loaded protein over 40  $\mu$ g or a very strong antibody)
  - 2. Quick rinse in TBST.
  - 3. Rinse 3 X's 10 minutes each in TBST.
  - 4. Re-block blot.
  - 5. Run a loading control after each stripping.

### **OR**

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0.5% Triton X-100	
0.5 mL Triton X-100	
95.5 mL ddH <sub>2</sub> O	

- 1. Incubate membrane in 0.5% Triton X-100 solution for 30 min. at room temp on a rocker.
- 2. Rinse 3 X's 10 minutes each in TBST.
- 3. Re-block blot.