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Biochemical Measures of Neuropathy - Western Blot Stripping

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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

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 70ºC. to strip. (10 min. for 20-40 µg protein and 15-20 min. for loaded protein over 40 µg 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

2

0.5% Triton X-100
0.5 mL Triton X-100
95.5 mL ddH_2O

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.