Aug 14, 2019

Biochemical Measures of Neuropathy - Western Blot Stripping

DOI
dx.doi.org/10.17504/protocols.io.3s3gngn

Eva Feldman[1]

[1]University of Michigan - Ann Arbor

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


Lili Liang

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.3s3gngn

External link: https://www.diacomp.org/shared/document.aspx?id=54&docType=Protocol

Protocol Citation: Eva Feldman 2019. Biochemical Measures of Neuropathy - Western Blot Stripping. **protocols.io https://dx.doi.org/10.17504/protocols.io.3s3gngn**

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

Created: June 05, 2019

Last Modified: August 14, 2019

Protocol Integer ID: 24123

Keywords: Biochemical Measures of Neuropathy, diabetic neuropathy, Western Blot Stripping, biochemical measures of neuropathy, neuropathy, increased levels of glucose overload mitochondria, glucose overload mitochondria, hyperglycemia, axons of the peripheral nervous system, oxidative stress, antioxidant capacity, excess glucose, diabetic complication, biochemical measure, metabolic change, peripheral nervous system, flow of excess glucose, cellular pathway

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 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.