AUG 14, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3sbgnan

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

Protocol Citation: Eva Feldman 2019. Biochemical Measures of Neuropathy -- Glutathione S-Transferase.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.3sbgnan>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jun 05, 2019

Last Modified: Aug 14, 2019

PROTOCOL integer ID:
 24099

Biochemical Measures of Neuropathy -- Glutathione S-Transferase

Eva Feldman¹

¹University of Michigan - Ann Arbor

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

Lili Liang

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

Keywords: Biochemical Measures of Neuropathy, diabetic neuropathy, Glutathiones-Transferase

MATERIALS

MATERIALS

⊗ HPLC-grade water **Fisher Scientific Catalog #W5-4**

⊗ 1-Chloro-2 4-Dinitrobenze CDNB **Sigma Aldrich Catalog #237329-10G**

⊗ Potassium phosphate Monobasic **Sigma Aldrich**

⊗ Potassium phosphate Dibasic **Sigma Aldrich**

⊗ EDTA **Fisher Scientific Catalog #16 004Y**

⊗ Reduced Glutathione **Sigma Aldrich Catalog #G6529-1G**

⊗ GST Control **Contributed by users**

Reagent Preparation:

Homogenizing Buffer: To 10 mL 100 mM potassium phosphate **pH to 6.5**, add **400 μ L** 50 mM EDTA. (Final concentration EDTA is 2mM)

Assay Buffer: Add 20 μ L Triton X-100 (0.1%) to 20 mL of 100 mM potassium phosphate buffer pH 6.5. Equilibrate to 25°C before using.

GST (Control): Solution of rat liver.

Glutathione: Add 6.15 mg to 2 mL HPLC H₂O. (10 mM)(**Final concentration in well 1 mM**) (Habig, The first enzymatic step in mercapturic acid formation. J.Biol. Chem. 249, 7130-7139 (1974))

CDNB: (Upstairs in fume hood.) Add 4 mg CDNB to 1mL ethanol. (20 mM) (Final concentration in well 1 mM)

Note:

Fisher Scientific, [RRID:SCR_008452](https://www.fishersci.com/catalog/rrid:SCR_008452)

Sigma-Aldrich, [RRID:SCR_008988](https://www.sigmaaldrich.com/catalog/rrid:SCR_008988)

Sample Preparation: Tissue

1. Label 2 sets of 1.5 ml micro-centrifuge tubes and 1 set of 0.5 ml tubes.
2. If not perfused at dissection, rinse tissue with ice cold homogenization buffer to remove RBC's and clots.
3. Sonicate the tissue on ~5 in 0.5–1 mL of cold homogenizing buffer per mg of tissue.

General Equation: $\mu\text{L Buffer} = \text{mg Tissue} \times 10$

4. Centrifuge at 10,000 x g for 15 minutes at 4°C.
5. Remove 15 μL supernatant and place in a 0.5 ml tube for protein analysis.
6. Transfer supernatant to a new labeled tube for assay and store on ice. Sample may be stored at – 80°C for at least one month.

Sample Preparation: Plasma

- 2 1. Collect blood in a tube containing an anticoagulant.
2. Centrifuge at 700-1000 x g for 10 min. at 4°C.
3. Place the top yellow plasma layer in a labeled 1.5ml micro-centrifuge tube.
4. Remove 15 μL supernatant and place in a 0.5 ml tube for protein analysis.

Erythrocyte Lysate:

- 3 1. Remove the white buffy layer (leukocytes) and discard.
2. Lyse the erythrocytes (RBC) in 4 times its volume of ice-cold **HPLC-grade water**.
3. Centrifuge at 10,000 x g for 15 min. at 4°C.
4. Place supernatant (erythrocyte lysate) in labeled 1.5ml micro-centrifuge tube. **Dilute prior to assay.**
5. Remove 15 μL supernatant and place in a 0.5 ml tube for protein analysis.

Performing Assay:

- 4 *Final volume of the assay is 200 μL in all wells*
 1. Turn on Multiskan and open file gst.sed and make sure the machine is set to 25°C.
 2. Add 20 μL of Assay Buffer to background wells, 20 μL GST control to the control wells and 20 μL sample to sample wells.
 3. Add 150 μL Assay Buffer to all wells.

4. Add 20 μ L of GSH to each well.
5. Initiate reactions by adding 10 μ L of CDNB to all wells as quickly as possible. **Note precise time the reaction is initiated.**
6. Place plate onto Multiskan holder and click **START** .
7. Read absorbance once every minute at 340 nm at least 5 minutes. ***Absorbance increase should be between 0.012 & 0.064/min. If not, dilute or concentrate sample. If initial reading is >0.7, dilute sample.***
8. From the sheet menu, select Process>Organize. Choose the appropriate data to organize (usually Measure1), and click **OK**. This re-arranges the data into columns.
9. Save organized data as an Excel file into the GTx data folder. Use the naming convention GTXXXX.xls, where XXXXXX is the date in yymmdd format.