Aug 14, 2019

Biochemical Measures of Neuropathy - Glutathione Reductase

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1 Works for me dx.doi.org/10.17504/protocols.io.3qsgmwe

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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:

EXTERNAL LINK

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

MATERIALS

NAME	CATALOG #	VENDOR
Cayman Glutathione Reductase Assay Kit	703202	Cayman Chemical Company
HPLC-grade water	W5-4	Fisher Scientific

MATERIALS TEXT

Reagent Preparation:

Assay Buffer (10X): Dilute 2 mL of Assay buffer concentrate with 18 mL of HPLC-grade water. Store at 4°C. Stable for 2 months.

Sample Buffer (10X): Dilute 2 mL of Sample buffer concentrate with 18 mL of HPLC-grade water. Store at 4°C. Stable for 1 month.

Glutathione Reductase (Control): Dilute 10 µL of supplied enzyme with 990 µL of diluted sample buffer. Keep on ice. Aliquot 70 µL into 0.5 mL centrifuge tubes and store at –20°C.

GSSG: Ready to use. Store at –20ºC.

NADPH: Reconstitute the number of vials required by adding 2 mL of HPLC-grade water to each vial and vortex. Each vial is enough reagent for 40 wells. Keep at 25ºC for assay. Store at 4ºC. Stable for 2 days.

Note:

Cayman Chemical (RRID:SCR_008945)

Thermo Fisher Scientific (RRID:SCR_008452)

Sample Preparation — Tissue:

1 1. Homogenize the tissue in 5–10 mL of cold assay buffer (i.e., 50 mM potassium phosphate, pH 7.5, 1 mM EDTA) per gram of tissue. (***General Equation:*** **µL Buffer = mg Tissue X 10***)*

2. Centrifuge at 10,000 x g for 15 minutes at 4°C.

3. Remove supernatant for assay and store on ice. Sample can be stored at –80°C for at least one month.

Performing Assay:

2 1. Turn on Multiskan and open file GRx.sed.

2. **Background Wells:** add 120 µL of Assay Buffer and 20 µL of GSSG to three wells.

3. **Positive Control Wells:** add 100 µL of Assay Buffer, 2 0µL of GSSG, and 20 µL of diluted GR (control) to three wells.

4. **Sample Wells:** add 100 µL of Assay Buffer, 20µL of GSSG, and 2 0µL of sample to three wells.

5. Initiate reactions by adding 50 µL of NADPH to all wells as quickly as possible. Note precise time the reaction is initiated.

6. Place plate onto Multiskan holder and click **START**.

7. Save raw data as an Excel file into the GRx data folder. Use the naming convention gxXXXX.xls, where XXXX is the date in mmdd format.

8. Select Process>Organize. Choose the appropriate data to organize (usually Measure1), then click **OK**. This rearranges the data into columns.

9. Save organized data as an Excel file into the GRx data folder. Use the naming convention grXXXXXX.xls, where XXXXXX is the date in mmddyy format.