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Biochemical Measures of Neuropathy - Glutathione Peroxidase

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1 Works for me dx.doi.org/10.17504/protocols.io.3qrgmv6

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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 Peroxidase Assay Kit	703102	Cayman Chemical Company

MATERIALS TEXT

Reagents & Supplies: HPLC-grade water

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 Peroxidase (Control): Dilute 10 µL of supplied enzyme with 490 µL of diluted sample buffer. Aliquot 70 µL into 0.5 mL centrifuge tubes and store at –20°C.

Co-Substrate Mixture: Reconstitute the number of vials required by adding 2 mL of HPLC-grade water to each vial and vortex. Each

vial will have enough reagent for 40 wells.

Cumen Hydroperoxide: Ready to use as supplied. Store at –20°C.

Note:

Cayman Chemical (RRID:SCR_008945)

Sample Preparation — Tissue:

1 1. Homogenize the tissue in 5–10 mL of cold buffer (i.e., 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT) 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 gpx.sed.

2. **Background Wells:** add 120 µL of Assay Buffer and 50µL of co-substrate mixture to three wells.

3. **Positive Control Wells:** add 100 µL of Assay Buffer, 50 µL of co-substrate mixture, and 20 µL of diluted GPx (control) to three wells.

4. **Sample Wells:** add 100 µL of Assay Buffer, 50 µL of co-substrate mixture, and 20 µL of sample to three wells.

5. Initiate reactions by adding 20 µL of cumen hydroperoxide to all wells being used 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 GPx 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 GPx data folder. Use the naming convention gxXXXXor.xls, where XXXX is the date in mmdd format.