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Biochemical Measures of Neuropathy - Lowry Protein Assay

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Protocol status: Working
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

- Reagent A (Store at 22 degrees celsius) **Bio-Rad Laboratories Catalog #**500-0113
- Reagent B (Store at 22 degrees celsius) **Bio-Rad Laboratories Catalog #**500-0114
- Reagent S (Store at 22 degrees celsius) **Bio-Rad Laboratories Catalog #**500-0115
- Standard (Lyophilized Bovine Plasma Gamma Globin (Store at 4 degrees celsius) **Bio-Rad Laboratories Catalog #**500-0005

Note:

Bio-Rad Laboratories RRID:SCR_008426

Performing the Assay:

1 1. Thaw samples on ice.

2. If samples contain detergent: add 20ul of reagent S to each ml for reagent A

3. Prepare standard as follows:

Standard #	Protein	Buffer
0.0	0 µl	15 µl
0.2	2.1 µl	12.9 µl
0.4	4.3 µl	10.7 µl
0.6	6.4 µl	8.6 µl
0.8	8.6 µl	6.4 µl
1.0	10.7 µl	4.3 µl
1.2	12.9 µl	2.1 µl
1.4	15µl	0 µl

4. Pipet 5 µl of standards and samples into plate.

5. Add 25 µl of reagent A to each well.

6. Add 200 µl reagent B to each well.

7. Place plate in reader and press **START.**

Reading the Plate – (wavelength 750 nm)

2 1. Wipe the bottom of plate to remove finger prints, dirt, etc.

2. Open ascent software to saved template. Place plate onto Multiskan holder and click **START**. Plate will shake for 5 seconds then incubate 15 min. then read plate at 750 nm.

3. Save raw data as an Excel file into the Lowry data folder. Use the naming convention LYXXXXXX.xls, where XXXXXX is the date in yymmdd format.

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

5. Save organized data as an Excel file into the LY data folder. Use the naming convention LYXXXXor.xls, where XXXX is the date in mmdd format.