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Biochemical Measures of Neuropathy - (H2) DCFDA

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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

DCFDA (50 µg m.wt. 535.76) **Molecular Probes**

HBSS **Molecular Probes**

DMSO (10 mg/mL) **Molecular Probes**

Reagent Preparation:

DCFDA: Dissolve 1 vial of DCFDA in 10 µL DMSO. (10mg/mL).

Note:

Molecular Probes (RRID:SCR_013318)

Assay Preparation:

1 Set up plate layout in AscentFL software. Choose DCFDA.sed and fill in your layout. Save your file as DCxxxxxx.sed with xxxxxx being the date in yy/mm/dd format.

2 Treat cells per experimental paradigm.

3 15-30 minutes prior to reading, add 3.23 µL of DCFDA stock to 15 mL media without serum.

4 Rinse cells with HBSS.

5 Place plate into Fluroskan holder and click **START**.

6 Take readings using 485 nm ex, 520 nm em filter pair.

7 Save sheet with .xls extension into the DCFDA data folder or your own folder. Use the naming convention **DC**XXXXXX.xls, where XXXX is the date in yymmdd format.