AUG 14, 2019

🌐 Biochemical Measures of Neuropathy - (H2) DCFDA

Eva Feldman¹

¹University of Michigan - Ann Arbor

Diabetic Complications Consortium

Tech. support email: rmcindoe@augusta.edu

Lili Liang

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.3n7gmhn

External link:

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

Protocol Citation: Eva Feldman 2019. Biochemical Measures of Neuropathy - (H2) DCFDA. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3n7gmhn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), 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 03, 2019

Last Modified: Aug 14, 2019

PROTOCOL integer ID:
23999

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, (H2) DCFDA

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](https://www.molecularprobes.com/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 **DCXXXXXX.xls**, where XXXX is the date in yymmdd format.