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Biochemical Measures of Neuropathy - DHE

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1 Works for me dx.doi.org/10.17504/protocols.io.3n8gmhw

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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
10 mM HEPES	View	Molecular Probes
150 mM NaCl		Molecular Probes
5 mM KCl	View	Molecular Probes
1 mM MgCl2	View	Molecular Probes
1.8 mM CaCl2	View	Molecular Probes
DHE		Molecular Probes
DMSO (10 mg/mL)	View	Molecular Probes

MATERIALS TEXT

Reagent Preparation:

HBSS (pH 7.4): Combine HEPES, NaCl, KCl, $MgCl_2$, and $CaCl_2$. pH to 7.4 then filter to sterilize. (may be stored at 22ºC for several months)

DHE: Dissolve 1 vial in 100 µL DMSO (10 mg/mL). Remove 10 µL, aliquot and freeze remainder. Protect from light and discard after thawing 3 times. Add the 10 µL to 1mL HBSS (0.1 mg/mL), then for the working dilution put 100 µL of diluted solution into 10 mL HBSS. (1 µg/mL=3µM)

Note:

Molecular Probes (RRID:SCR_013318)

1 Open Fluoroskan and choose open under the file menu. Scroll down and select DHE.sed. Set up your plate layout. Save your layout as DHxxxxxx.sed with xxxxxx being yy/mm/dd.

2 *Treat cells per experimental paradigm.

Note: *As an alternative, cells can be pre-loaded with DHE- do steps 3, 4, 5, 2, then 5.

3 15 minutes prior to reading, ***gently*** rinse cells once with HBSS.

4 Apply 3 µMDHE in HBSS and leave on for 15 minutes.

5 Rinse cells and add HBSS.

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

7 Take readings using 485 nm ex, 612 nm em filter pairs for ethidium and 355 nm ex, 430 nm em for DHE.

8 Save **both** sheets with .xls extension into the ***DHE*** data folder or your own folder. Use the naming convention ***DH***XXXX.xls, where XXXX is the date in mmdd format and add a **r** for the red reading and **b** for the blue reading.