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Biochemical Measures of Neuropathy - Genotyping

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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

- 10X PCR Buffer **Life Technologies Catalog #**10966-034
- 50 mM MgCl2 **Life Technologies Catalog #**10966-034
- 10 mM dNTPs **Life Technologies Catalog #**10297-018
- Taq DNA Polymerase (5 U/µL) **Life Technologies**
- 0.5 mL thin-walled tubes **NEN Life Science Products Inc Catalog #**LS-9350-X
- Forward primer (10 µM)
- Reverse primer (10 µM)
- DNA Template (200 ng/µL)
- Sterile deionized H2O

Note:

Life Technologies (RRID:SCR_008817)

PCR Amplification:

1 Prepare a Master Mix containing the following volumes of reagents:

Reagent	µL
10X PCR Buffer	2.5
50 mM $MgCl_2$	1.25
dNTPs	2.5
M13 Forward Primer	1.0
M13 Reverse Primer	1.0
Taq DNA Polymerase	0.2
DNA Template	2.0
Sterile dd-H2O	14.55
Total Volume =	25.00

2 Into each 0.5mL thin-walled tube, pipette 23.0-µL of Master Mix.

3 Pipet 2.0 µL of DNA template (200 ng/µL) into thin-walled tube. Mix gently, and spin down.

4 Amplify using the following program:

94°C, 5 min. → 94°C, 1 min. → 64°C, 1 min. → 72°C, 1.5 min. → 72°C, 10 min. → 4°C, soak
Repeat 35 times

5 Verify PCR product by separation on 2% agarose.

6 Store tubes at –20°C until needed. If plates will be used within a few days they may be stored at 4°C.