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Biochemical Measures of Neuropathy - Aconitase

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1 Works for me dx.doi.org/10.17504/protocols.io.3n4gmgw

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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
BIOXYTECH® Aconitase-340	21041	
Sodium Citrate	S-4641	Sigma Aldrich
Tris-HCl		Fisher Scientific

MATERIALS TEXT

Kit: OxisResearch, Bioxytech Aconitase-340, Catalog No. 21041 (store at 4ºC)

Reagent Preparation:

0.2mM Sodium Citrate in 50 mM Tris-HCl pH 7.4: 3.15g in 300mL deionized H_2O, add 23.5mg Sodium Citrate. pH to 7.4 then bring up to 400mL.

NADP Reagent: Just prior to use, reconstitute the NADP with 5.5 mL deionized H_2O.

Enzyme: Reconstitute with 10 mL deionized water. Store at 4°C.

Note:

Sigma-Aldrich, RRID:SCR_008988

Fisher Scientific, RRID:SCR_008452

Sample Preparation — Tissue

1 1. Weigh tissue sample.

2. Mince tissue.

3. Homogenize at 1% (w/v) in ice cold 0.2mM sodium citrate for 15-20 seconds. (new protocol calls for sodium citrate in 50mM Tris-HCl, pH 7.4).

4. Centrifuge at 800 x g for 10 minutes at 4°C.

5. Remove 25µl supernatant for protein assay.

6. Remove supernatant and store on ice or freeze –80°C until use.

Performing Assay:

2 1. Thaw homogenate on ice.

2. Fill in layout on computer and save as acxxxxxx.sed where xxxxxx is the date in yyddmm format.

3. Sonicate for 20 seconds.

4. Dilute the tissue extract 10µl extract into 190µl (500µg/ml) assay buffer.

5. Add 50µl of sample to each well in triplicate. (Do 1:1 and 1:20 dilutions.)

6. Add 50µl assay buffer to blank well in triplicate.

7. Add 50µl of Substrate to each well.

8. Add 50µl of Enzyme to each well.

9. Add 50µl of NADP to each well.

10. Mix using a pipet

Reading the Plate:

3 1. Record absorbance change at 340nm for 5 minutes at 37ºC.

2. Turn on Multiskan and open your saved file from above.

3. Place plate onto Multiskan holder and click **START**.

4. Save raw data as an Excel file into the acx data folder. Use the naming convention acXXXX.xls, where XXXX is the date in mmdd format.

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

6. Save organized data as an Excel file into the acx data folder. Use the naming convention acXXXXor.xls, where XXXX is the date in mmdd format.