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NADH Oxidase Activity

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

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ABSTRACT

Summary:

Describes assay to quantitate NADH Oxidase activity from tissues.

Diabetic Complications:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=36&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
50mM Tris	15504-012	Gibco - Thermo Fischer
50mM MES buffer	View	Sigma Aldrich
150µM NADH	N 6879	Sigma Aldrich

MATERIALS TEXT

Reagent Preparation:

Tris-MES buffer (pH 7.0):
NADH

Tris-MES buffer (pH 7.0): Prepare 50mM Tris buffer solution and pH to 7.0 with 50mM MES. 50mM Tris - 302.85mg Tris in 50ml deionized H_2O. 50mM MES - 319.89mg MES in 50ml deionized H_2O.

NADH: Prepare 600µM solution. 2.55mg in 6mL de-ion H_2O, enough for whole plate.

Note:

Fisher Scientific, RRID:SCR_008452
Sigma-Aldrich, RRID:SCR_008988

Sample Preparation:

1 Turn on Multiskan, set temp to 37°C and set up plate layout.

2 Sonicate tissue on ice in 20mM PB pH 7.4 with PMSF inhibitor or thaw prepared samples on ice.

3 Remove 25µL for protein analysis.

4 Prepare NADH, enough for whole plate.

5 Dilute samples 1:5 with de-ionized H_2O.

6 Using a clear plate: Add 50µL sample to wells and 50µL diluted sample to wells in duplicate.

7 Add 50µL buffer to 3 wells for blanks for positive control.

8 Add 100µL Tris-Mes to each sample and blanks.

9 For negative control add 200µL Tris-Mes to 3 wells.

10 Place plate in Multiskan and add 50µL 600µM NADH to the sample and positive blanks.
Do not add NADH to the 3 negative control wells.

11 Press start and read at 340nm for 10 minutes @ 1 minute intervals.

12 Save raw data as an Excel file into the NADHx data folder. Use the naming convention NAXXXX.xls, where XXXX is the date in mmdd format.