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 We use this protocol and it's working

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NADH Oxidase Activity

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ABSTRACT

Summary:

Describes assay to quantitate NADH Oxidase activity from tissues.

Diabetic Complications:

Cardiovascular

Nephropathy

Neuropathy

Pediatric Endocrinology

Retinopathy

Uropathy

Wound-Healing

Keywords: NADH Oxidase activity, Cardiovascular, Nephropathy, Neuropathy, Pediatric Endocrinology, Retinopathy, Uropathy, Wound-Healing

MATERIALS

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50mM Tris Gibco - Thermo Fischer Catalog #15504-012

50mM MES buffer Sigma Aldrich

150μM NADH Sigma Aldrich Catalog #N6879

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 H₂O. 50mM MES - 319.89mg MES in 50ml deionized H₂O.

NADH: Prepare 600μM solution. 2.55mg in 6mL de-ion H₂O, 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 H₂O.
- 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.