NADH Oxidase Activity

Version: 1
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Summary
Reagents and Materials
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Reagent Preparation
Tris-MES buffer (pH 7.0):
NADH

Summary: Describes assay to quantitate NADH Oxidase activity from tissues.

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
50mM Tris		Gibco	15504-012
50mM MES buffer		Sigma	
150µM NADH		Sigma	N 6879

Protocol:

Sample Preparation:

1. Turn on Multiskan, set temp to 37^0 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.

Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

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.