NADH Cytochrome C Reductase

References-
1) Sottocasa, G. L. Journal of Cell Biology-32: p415-4
2) Ernister, L. J. Biol. Chem.238:1124
3) Tahmoush et al-Muscle & Nerve 20: 1219-1224 1997.
4) Reichmann et al- Arch of Neurology 43: 957-961

Principle- Absortion of Cytochrome C at 550 nm changes with it's oxidation state.
The activity of NADH cytochrome c reductase is measured spectrophotometrically by following the reduction of cytochrome c at 550nM.The mitochondrial portion of this enzyme, unlike the external portion, is sensitive to rotenone. The enyme is measured with and without rotenone, and the difference is reported as intramitochondrial, or rotenone sensitive enzyme activity. (1 mM Cytochrome C =20 per Haller lab)

Solutions– make the following stock solutions-

1. 1M Potassium Phosphate pH 7.5
Pottasium Phosphate Dibasic (Sigma P5504) FW 228.2 .
Weigh 22.8 gms and dissolve in app. 80 mls Milliq water. Ph to 7.5 with phosphoric acid. (**CORROSIVE .**WEAR GLOVES, LAB COAT AND SAFETY GLASSES)Dilute to final volume of 100 mls. Store at 4°C for up to one year. Discard if any growth, or floating material is present.

2. 100 mM KCN –
Fisher P –226 FW 65.0
Weigh **6.5 mg** on day of assay, and dissolve in **1 ml** of MilliQ water. Keep on ice.
(POISON- WEAR GLOVES, MASK , AND LAB COAT)

3. Rotenone- Weigh 2 mgs on day of assay and dissolve in 1 ml ethanol. Keep at 30°C.

4. 10 mM Cytochrome C-

Sigma C-7752 FW 12384. Weigh 123.8 mgs and dissolve in 1 ml MilliQ water. Store at -80°C for up to 1 year.

5. 10 mM NADH -
Sigma- N6785- 10mg/vial. Make fresh. Add 950ul H2O. Discard leftovers.
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Assay Reagent-

	Final Conc.	Stock Conc.	/10 ml
Cytochrome C	0.1mM	10mM	100µl
KCN	0.3mM	100mM	30µl
KPO4	50mM	1M	0.5ml

Assay Protocol-

Turn on Spectrophotometer (UNICO UV2100)
Change wavelength to 550 nm. Zero with air.
Mix the following in the cuvette. **Do 1 sample (4 cuvettes)at a time**.
Add rotenone to second set, and mix well.
Add homogenate and mix well.
Start timer on addition of NADH.

OD at 550 nM

#	Assay reagent	homog. medium	1:10 homogenate	Rotenone	NADH	1'	2'	3'	4'	5'
1.	0.5 ml	5µl			5µl					
2.	0.5 ml	5µl			5µl					
3.	0.5 ml	5µl		1µl	5µl					
4.	0.5 ml	5µl		1µl	5µl					
5.	0.5 ml		5µl		5µl					
6.	0.5 ml		5µl		5µl					
7.	0.5 ml		5µl	1µl	5µl					
8.	0.5 ml		5µl	1µl	5µl					
9.	0.5 ml		5µl		5µl					
10	0.5 ml		5µl		5µl					
11.	0.5 ml		5µl	1µl	5µl					
12.	0.5 ml		5µl	1µl	5µl					
13.	0.5 ml		5µl		5µl					
14.	0.5 ml		5µl		5µl					
15.	0.5 ml		5µl	1µl	5µl					
16.	0.5 ml		5µl	1µl	5µl					

Calculations- µmoles/gm/min= $\frac{\text{net sample OD per minute}}{\text{0.5 mg}} * \frac{\text{final volume(0.501 ml)}}{\text{20 } (\Delta\varepsilon \text{ mM})}$

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