

Cytochrome Oxidase

References-

- 1) Wharton et. al. Methods of Enzymology Vol 10:245-250
- 2) Smith et. Al. Methods of Biochemical Analysis Vol 2 :427
- 3) DiMauro et. al Neurology 30:795

Principle- Absorption of Cytochrome C at 550 nm changes with its oxidation state. Reduced Cytochrome c is prepared, checked (550/565 >8) and frozen at -80°C. Its oxidation by the enzyme is a measure of COX activity. The difference in the extinction coefficient ($\Delta\epsilon$ mM) is taken as 20 at 550nm. (See Dr. Haller protocol and reference.) (1 mM Cytochrome C =20)

This is a biphasic reaction with an initial burst of activity followed by a slower reaction rate. Activity is measured 1 cuvette at a time.

Solutions- make the following stock solutions-

1. 100 mM Phosphate buffer pH 7.0-

0.06M Potassium Phosphate Dibasic (Sigma P5504) FW 228.2

Weigh 1.369 gms

0.04M Potassium Phosphate Monobasic (Fisher P-284) FW 136.09

Weigh 0.54 gm

Dissolve in MilliQ water to a final volume of 100mls.

Store at 4°C for up to 1 year. Do not use if solution gets turbid.

2. 1% reduced Cytochrome C

Sigma C-7010 Horse heart type IIA MW 12384 80% pure.

Compensate for the purity by weighing 0.125 gms and dissolve in 1.0 ml cold 0.01M phosphate buffer. (Dilute from 0.1M stock -1.0 ml/10ml final volume.) Add a few mgs of (1 or 2) of Ascorbic acid (Sigma A-5960) and stir till dissolved. Dialyse for 2-3 hrs against cold 0.01M phosphate buffer to remove excess ascorbic acid. Immediately freeze in 1 ml aliquots at -80°C in tubes wrapped with aluminum foil. Store for 1 year.

Assay Protocol-

Turn on Spectrophotometer (UNICO UV2100)

Change wavelength to 550 nm. Zero with air.

Mix the following in the cuvette. **Do 1 cuvette at a time.**

Start timer on addition of Cytochrome C

OD at 550 nM

#	0.01M phosphate ph 7.0	homog. medium	1:10 homogenate	0.8mM reduced Cyt C	15"	30"	45"	1'
1.	0.95 ml	5µl		50µl				
2.	0.95 ml	5µl		50µl				
3.	0.95 ml		5µl	50µl				
4.	0.95 ml		5µl	50µl				
5.	0.95 ml		5µl	50µl				
6.	0.95 ml		5µl	50µl				
7.	0.95 ml		5µl	50µl				
8.	0.95 ml		5µl	50µl				
9.	0.95 ml		5µl	50µl				
10.	0.95 ml		5µl	50µl				
11.	0.95 ml		5µl	50µl				
12.	0.95 ml		5µl	50µl				

Calculations- $\mu\text{moles/gm/min} = \frac{\text{net sample OD per minute} * \text{final volume}(1.005 \text{ ml})}{0.5 \text{ mg} \quad 20 (\Delta\epsilon \text{ mM})}$

The normal range for this assay is $\geq 1.2 \mu\text{moles/gm/min}$

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Approved Dr. Alan Pestronk