

Ubiquinol-ferricytochrome c oxidoreductase

References-

- 1) Kwong, L. And Sohal, R. Archives of Biochemistry and Biophysics: 373, p16-22 2000
- 2) Trounce et. Al Methods in Enzymology, Vol 264 p-484-509

Principle- Absorption of ferricytochrome c increases with its reduction.

The activity of ubiquinol-ferricytochrome c is measured spectrophotometrically by following the reduction of ferri-cytochrome c at 550nm.

(1 mM Cytochrome C = 20 reference -Haller)

Solutions- make the following stock solutions-

1. 1M Potassium Phosphate pH 7.5

Potassium 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. 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.

4. 100 mM EDTA

Prepare by weighing 3.72 gms EDTA sigma ED2SS, MW 372.2. Dissolve in a final volume of 100 ml with MilliQ water. Store at 4°C.

5. 2 mg/ml Rotenone

Measure 2 mgs Rotenone (Sigma R -8875). **WARNING- TOXIC. Wear Gloves and protective lab coat when working with this material.** Dissolve in 1 ml Ethyl Alcohol 200 proof. Keep solution at 37°C.

6. Decylubiquinone (DBH2)

Make fresh on day of use. Add a small crystal of potassium borohydride to 50 µl of 10 mM decylubiquinone (3.22 mgs/ml Sigma D-7911 FW 322.4) in Ethanol. Add 5 µl aliquots of 0.1 M HCl with gentle mixing until the yellow solution becomes colorless. Transfer the DBH2 to a fresh tube, and add 5 µl of 1 M HCl. Record the final DBH2 concentration. Store on ice while performing assays, and if the color changes back to yellow before use, repeat the reduction procedure.

Assay Reagents-

	Final Conc.	Stock Conc.	/25 ml
KPO4	25 mM	1M	0.625 ml
KCN	2 mM	100mM	100µl
Rotenone	4µg/ml	2 mgs/ml	50µl
EDTA	1 mM	100 mM	0.25 ml
Cyt C	50µM	10 mM	0.125 ml

Assay Protocol-

Turn on Spectrophotometer (UNICO UV2100)

Change wavelength to 550 nm. Zero with air.

Mix the following in the cuvette. **Do 4 cuvettes at a time.** Mix well.

Add homogenate and mix well.

Start timer on addition of 50 µM DBH2.

OD at 550 nM

#	Assay reagent	homog. medium	1:10 homogenate	DBH2	1'	1.30'	2'
1.	1 ml	5µl		5µl			
2.	1 ml	5µl		5µl			
3.	1 ml			5µl			
4.	1 ml			5µl			
5.	1 ml		5µl	5µl			
6.	1 ml		5µl	5µl			
7.	1 ml		5µl	5µl			
8.	1 ml		5µl	5µl			
9.	1 ml		5µl	5µl			
10.	1 ml		5µl	5µl			
11.	1 ml		5µl	5µl			
12.	1 ml		5µl	5µl			
13.	1 ml		5µl	5µl			
14.	1 ml		5µl	5µl			
15.	1 ml		5µl	5µl			
16.	1 ml		5µl	5µl			

Calculations-

$$\mu\text{moles/gm/min} = \frac{\text{net sample OD per minute}(2'-1.30' \times 2) \times \text{final volume (1.015 ml)}}{0.5 \text{ mg} \quad 20 (\Delta\epsilon \text{ mM})}$$

Updated 2.27.09 RC

Approved Dr. Alan Pestronk