COX ASSAY

Extraction

Wash cells with cold PBS x2. Place 1.0mL cold PBS on plate and scrape cells. Place in eppendorf tube and centrifuge at 10,000g at 4°C for one minute. Remove supernatant and resuspend pellet in extraction buffer. For a confluent 100mm plate use ~100-200µL. Keep on ice. Homogenize cells with motorized pestle for 15 seconds. Use homogenate within the next few hours. Citrate synthase appears more sensitive to time so perform this assay first.

Extraction buffer TRA-EDTA pH7.4:

Triethanolomine 50mM (9.28g/L)
EDTA 1.0mM (0.2924g/L)
Store at 4°C

Citrate Synthase

Reagent mix per mL	
Assay buffer	500µL
Acetyl – CoA	20
Ellman's reagent	20
Triton X-100 10%	10
H_2O	500

Heat reagent mix to 30°C in water bath.

per well	
Homogenate	variable (~5-10µL)
Oxaloacetic acid	7µL (0 for blank)
Reagent Mix	200µL

Measure increase in absorbance at 412 nm at 30°C

Reagents
-Assay buffer- Tris 200mM (24.24g/L) pH 7.4 with HCl. Store at 4°C
-Acetyl CoA (Roche 101907)- 8.3mg/1.0mL (5.5mL per 50mg bottle). Prepare in Milli Q H_2O.Store at –20°C indefinitely
-Ellman's (DTNB) (Sigma D8130)- 4.0mg /1.0mL. Prepare 40mg in 10mL 95% ETOH. Store at –20°C indefinitely
-Oxaloacetic Acid (SigmaO-4126) 1.3mg/1.0mL. Prepare 10mL and pH to 7.4 with KOH. Aliquot and store at–20°C for one month.
-Triton X-100 10% in H_2O v/v. Store at 4°C or –20°C indefinitely.

Cytochrome C Oxidase

Reagent Mix per mL

Assay buffer	500µL
Cytochrome C	variable
Dodecylmaltoside 10%	10µL
H_2O	500µL

Heat reagent mix to 30°C in water bath.

per well

Homogenate	variable (~5-10µL)
Reagent Mix	200µL

Measure decrease in absorbance at 550nm at 30°C

Reagents

-Assay buffer Potassium Phosphate 100mM pH7.0
K2HP04 10.71g/L H_2O
KH2P04 5.24g/L H_2O
Store at 4°C

Reduced Cytochrome C- cytochrome c (SigmaC2506) 123.5mg/mL in 50mM potassium phospate buffer pH 7.0 . Add 7.5 mg/mL sodium dithionite and incubate in the dark on ice for 5 minutes. Purify on a 10K MWCO spin filter (Millipore UFV2BGC10) by spinning at 2000g for ~one hour, adding more buffer every 15 minutes and mixing with the cytochrome c. Check the reduction of the cytochrome c by measuring the ratio of absorbance at 550/565nm. It should be >6. To calculate the concentration of reduced cytochrome c (19.6)

n-Dodecylmaltoside- 10% w/v in H_2O. Store at 4°C or –20°C indefinitely.