Enzymatic Assay of CYTOCHROME OXIDASE
(EC 1.9.3.1)

PRINCIPLE:

$$\text{Cytochrome c (reduced)} + 2H^{+} + \tfrac{1}{2}O_2 \xrightarrow{\text{Cytochrome Oxidase}} \text{Cytochrome c (oxidized)} + H_2O$$

CONDITIONS: T = 37°C, pH = 7.0, A_{550nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 37°C with 1 M KOH.)

B. 10 mM Potassium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 2 liters in deionized water using Reagent A. Adjust to pH 7.0 at 37°C with 1 M KOH or 1 M HCl.)

C. 1.0% (w/v) Cytochrome c, Reduced, Solution
(Prepare 10 ml by dissolving 100 mg of Cytochrome c, Sigma Prod. No. C-2506, in 8 ml of Reagent B. Reduce the Cytochrome c by adding 3 - 5 mg of L-Ascorbic Acid, Sodium Salt, Sigma Prod. No. A-7631. The excess ascorbate is removed by dialyzing against Reagent B for 18 - 24 hours at 0 - 4°C with three changes of buffer. Remove from dialysis and bring up to a final volume of 10 ml with Reagent B. This Cytochrome c remains reduced for several months if stored at 0 - 4°C.)

D. 100 mM Potassium Ferricyanide Solution[1]
(Prepare 10 ml in Reagent A using Potassium Ferricyanide, Sigma Prod. No. P-8131. **HANDLE WITH EXTREME CAUTION**.)

E. 250 mM Sucrose Solution with 1% (v/v) Tween 80
(Prepare 50 ml in Reagent A using Sucrose, Sigma Prod. No. S-9378, and Polyoxyethylenesorbitan Monooleate, Tween 80, Sigma Prod. No. P-1754.)

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REAGENTS: (continued)

F. Cytochrome Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 mg solid/ml (0.02 mg protein/ml) of Cytochrome Oxidase in cold Reagent E.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent B (Buffer)	2.70	2.70
Reagent C (Cytochrome c, Reduced)	0.20	0.20
Reagent D (Potassium Ferricyanide)	------	0.10

Mix by inversion and equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.10	------

Immediately mix by inversion and monitor the decrease in A_{550nm} for 10 minutes, recording the A_{550nm} values at one minute intervals.

CALCULATIONS:

$$\Delta E = E_t - E_f$$

E_t = the A_{550nm} value at the time points 0 through 10 minutes for the test
E_f = the A_{550nm} value at the 10 minute time point for the blank

Plot Ln(ΔE) *vs* Time. Determine the slope (M). The activity is calculated using the following equation:

$$\text{Units/mg solid} = \frac{(M)}{(\text{mg solid/RM})}$$

M = Slope of Ln (DE) versus time
RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of reduced cytochrome c to oxidized cytochrome c (using 0.5 µmole of oxygen) per

minute at pH 7.0 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 13 mM potassium phosphate, 0.07% (w/v) cytochrome c, 8.3 mM sucrose, 0.03% (v/v) Tween 80, and 0.01 mg solid cytochrome oxidase.

REFERENCE:

Wharton, D. C. and Tzagoloff, A. (1967) *Methods in Enzymology* **10**, 245-250

NOTES:

1. Potassium Ferricyanide is included in the blank only in order to completely oxidize the reduced cytochrome c.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.