

**Enzymatic Assay of CYTOCHROME OXIDASE
(EC 1.9.3.1)**

PRINCIPLE:

Cytochrome c (reduced) + 2H⁺ + ½O₂ $\xrightarrow{\text{Cytochrome Oxidase}}$ Cytochrome c (oxidized) + H₂O

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 Solution¹
(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.)

**Enzymatic Assay of CYTOCHROME OXIDASE
(EC 1.9.3.1)**

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:

	<u>Test</u>	<u>Blank</u>
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
 $A_{550\text{nm}}$ for 10 minutes, recording the $A_{550\text{nm}}$ values at one
minute intervals.

CALCULATIONS:

$$\Delta E = E_t - E_f$$

E_t = the $A_{550\text{nm}}$ value at the time points 0 through 10 minutes
for the test

E_f = the $A_{550\text{nm}}$ value at the 10 minute time point for the
blank

Plot $\ln(\Delta E)$ 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 $\ln(\Delta E)$ 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.

**Enzymatic Assay of Cytochrome Oxidase
(EC 1.9.3.1)**

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.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.