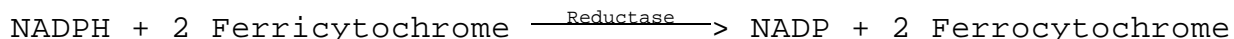


**Enzymatic Assay of CYTOCHROME P450 REDUCTASE
(EC 1.6.2.4)**

PRINCIPLE:

Cytochrome P450



CONDITIONS: T = 30°C, pH = 7.7, A_{550nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1 M Potassium Phosphate Buffer, pH 7.7 at 30°C
(Prepare 10 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.7 at 30°C with 1 M KOH.)
- B. 100 mM Ethylenediaminetetraacetic Acid
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- C. 300 mM Potassium Phosphate Buffer with
0.1 mM Ethylenediaminetetraacetic Acid, pH 7.7 at 30°C
(Working Buffer)
(Prepare 20 ml by combining 6 ml of Reagent A and 0.02 ml of Reagent B. Dilute to 20 ml with deionized water.)
- D. 0.036 mM Cytochrome C (Cyt C)
(Prepare 10 ml in Reagent C using Cytochrome C, Sigma Prod. No C-2506.)
- E. 1 mM Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form (NADPH)
(Prepare 5 ml in deionized water using Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- F. 0.05% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepared 5 ml in Reagent C using Albumin, Bovine, Sigma Prod. No. A-4503.)

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REAGENTS: (continued)

G. Cytochrome P450 Reductase Enzyme Solution
(Immediately before use, prepare a solution containing
0.1-0.25 unit/ml of Cytochrome P450 Reductase in cold
Reagent F.)

PROCEDURE:

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

	Test	Blank
Reagent D (Cyt C)	0.92	0.92
Reagent G (Enz Soln)	0.08	-----
Reagent F (Enz Dil)	-----	0.08

Equilibrate to 30°C. Then add:

Reagent E (NADPH)	0.10	0.10
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Immediately mix by inversion and record the increase in
 $A_{550\text{nm}}$ for 2 minutes. Obtain the $r A_{550\text{nm}}$ /minute using the
maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{550\text{nm}}/\text{min Test} - r A_{550\text{nm}}/\text{min Blank})(1.1)(\text{df})}{(21)(0.08)}$$

1.1 = Total volume (in milliliters) of assay

df = Dilution factor

21 = Difference in the millimolar extinction
coefficient

between oxidized and reduced cytochrome c.

0.08 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will catalyze the reduction of 1.0 μmole of
cytochrome C by NADPH per minute at pH 7.7 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 273 mM potassium phosphate, 0.09 mM ethylenediaminetetraacetic acid, 0.03 mM cytochrome c, 0.09 mM nicotinamide adenine dinucleotide phosphate, reduced form, 0.004% (w/v) bovine serum albumin, and 0.008-0.02 unit cytochrome P450 reductase.

REFERENCES:

Masters, B.S.S., Williams, C.H., Kamin, H. (1967) *Methods in Enzymology*, X, 565-573

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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