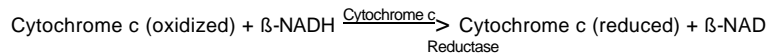


## Enzymatic Assay of CYTOCHROME c REDUCTASE (EC 1.6.99.3)

### PRINCIPLE:



#### Abbreviations:

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH = 8.5,  $A_{550\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 300 mM Glycylglycine Buffer, pH 8.5 at 25EC (Gly Gly)  
(Prepare 100 ml in deionized water using Gly-Gly, Hydrochloride, Prod. No. G-1127. Adjust pH to 8.5 at 25°C with 1 M  $\text{NH}_4\text{OH}$ .)
- B. 1% (w/v) Cytochrome c Solution  
(Prepare 10 ml in deionized water using Cytochrome c from Horse Heart, Prod. No. C-2506.)
- C. 7.05 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form ( $\beta$ -NADH)  
(Dissolve the contents of one vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Stock No. 340-105, in 1 ml of Reagent A.)
- D. 20 mM Potassium Bicarbonate Buffer, pH 8.5 at 25EC ( $\text{KHCO}_3$ )  
(Prepare 100 ml in deionized water using Potassium Bicarbonate, Prod. No. P-9144. Adjust pH 8.5 at 25°C with 1 M HCl.)
- E. Cytochrome c Reductase Enzyme Solution (CCR)  
(Immediately before use, prepare a solution containing 0.05 - 0.25 units/ml of Cytochrome c Reductase in cold Reagent D.)

**Enzymatic Assay of CYTOCHROME c REDUCTASE  
(EC 1.6.99.3)**

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	2.50	2.50
Reagent A (Gly Gly)	0.20	0.20
Reagent B (Cytochrome c)	0.10	0.10
Reagent C ( $\beta$ -NADH)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the  $A_{550nm}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	-----
Reagent D ( $KHCO_3$ )	-----	0.10

Immediately mix by inversion and record the increase in  $A_{550nm}$  for approximately 5 minutes. Obtain the  $r_{A_{550nm}}$  / minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{(r_{A_{550nm}}/\text{min Test} - r_{A_{550nm}}/\text{min Blank})}{(21.0) (\text{mg enzyme/ml RM})}$$

21.0 =  $\epsilon$  millimolar extinction coefficient between oxidized and reduced cytochrome c at pH 8.5  
RM = Reaction Mix

**UNIT DEFINITION:**

One unit will reduce 1.0  $\mu$ mole of oxidized cytochrome c per minute at pH 8.5 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 3 ml reaction mix, the final concentrations are  
30 mM glycylglycine, 0.033% cytochrome c,  
0.24 mM  $\beta$ -NADH and 0.005 - 0.025 units of cytochrome c reductase.

**Enzymatic Assay of CYTOCHROME c REDUCTASE  
(EC 1.6.99.3)**

**NOTE:**

1. All products 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.**