Enzymatic Assay of CYTOCHROME c REDUCTASE

(EC 1.6.99.3)

PRINCIPLE:

$$\text{Cytochrome c (oxidized)} + \text{ß-NADH} \xrightarrow[\text{Reductase}]{\text{Cytochrome c}} \text{Cytochrome c (reduced)} + \text{ß-NAD}$$

Abbreviations:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 8.5, A_{550nm}, 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 NH_4OH.)

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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form (ß-NADH)
(Dissolve the contents of one vial of ß-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 ($KHCO_3$)
(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.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	2.50	2.50
Reagent A (Gly Gly)	0.20	0.20
Reagent B (Cytochrome c)	0.10	0.10
Reagent C (ß-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 ΔA_{550nm}/ minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will reduce 1.0 µmole 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 ß-NADH and 0.005 - 0.025 units of cytochrome c reductase.

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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.