Enzymatic Assay of CERULOPLASMIN

PRINCIPLE:

N,N-Dimethyl-p-phenylenediamine + H_2O $\xrightarrow{\text{Ceruloplasmin}}$ Oxidized product

CONDITIONS: T = 37°C, pH = 5.5, A_{550nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Sodium Acetate Buffer, pH 5.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No S-8625. Adjust to pH 5.5 at 37°C with 1 M Acetic Acid.[1])

B. 153 mM N,N-Dimethyl-p-phenylenediamine Solution (DPD)[2]
(Prepare 10 ml in deionized water using N,N-Dimethyl-p-phenylenediamine, Monohydrochloride, Sigma Prod. No. D-5129. **PREPARE FRESH. STORE ON ICE. KEEP FROM LIGHT.**)

C. 100 mM Sodium Chloride Solution (Enzyme Diluent)
(Prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

D. Ceruloplasmin Solution (Ceruloplasmin)
(Immediately before use, prepare a solution containing approximately 30 units/ml of Ceruloplasmin in cold Reagent C.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.00	2.00
Deionized Water	0.80	0.80
Reagent C (Enzyme Diluent)	------	0.10
Reagent D (Ceruloplasmin)	0.10	------

PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Monitor the A_{550nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent B (DPD)	0.10	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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{550nm}/\text{min Test} - \Delta A_{550nm}/\text{min Blank})(3)(df)}{(0.01)(7)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
0.01 = Change in Absorbance at 550 nm (Unit Definition)
7 = Conversion Factor to published Unit Definition of a 7 ml reaction volume[3]
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit is arbitrarily defined as that amount of "Oxidase" which will cause a ?A_{550nm} of 0.01 per minute using N,N-dimethyl-p-phenylenediamine as substrate at pH 5.5 and 37°C, in a 7 ml reaction volume.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 133 mM sodium acetate, 5.1 mM N,N-dimethyl-p-phenylenediamine hydrochloride, 3.3 mM sodium chloride and 3.0 units ceruloplasmin.

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REFERENCE:

Curzon, G. and Vallet, L. (1960) *Biochem. J.* **74**, 279-287.

NOTES:

1. Do NOT use HCl for adjusting the pH. The chloride ion is an inhibitor of human ceruloplasmin and the concentration must be kept constant. The NaCl is necessary for diluting the enzyme solution.

2. The solution is stable for approximately 2-3 hours. A fresh solution should be prepared if an increase in absorbance is seen in the Blank.

3. This assay is based on the cited reference.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.