

**Enzymatic Assay of TYRAMINE OXIDASE  
(EC 1.4.3.4)**

**PRINCIPLE:**

Tyramine + H<sub>2</sub>O + O<sub>2</sub>  $\xrightarrow{\text{Tyramine Oxidase}}$  p-HPA + NH<sub>3</sub> + H<sub>2</sub>O<sub>2</sub>

2H<sub>2</sub>O<sub>2</sub> + 4-AAP + Phenol  $\xrightarrow{\text{POD}}$  Quinoneimine Dye + 2H<sub>2</sub>O

Abbreviations used:

p-HPA = p-Hydroxyphenylacetaldehyde

4-AAP = 4-Aminoantipyrine

POD = Peroxidase

**CONDITIONS:** T = 37°C, pH 7.5, A<sub>480nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 20 mM Potassium Phosphate Buffer with 2.12 mM Phenol, 1.48 mM 4-Aminoantipyrine, and 0.1 mM Tyramine, pH 7.5 at 37°C (Reaction Mix)  
(Prepare 20 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Phenol, Sigma Prod. No. P-3653, 4-Aminoantipyrine, Sigma Prod. No. A-4382, and Tyramine, Free Base, Sigma Prod. No. T-7255. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 100% (v/v) Ethanol (ETOH)  
(Use 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)
- C. Peroxidase (POD)  
(Use Peroxidase, Sigma Prod. No. P-8250.)
- D. 20 mM Potassium Phosphate Buffer, pH 7.5 at 37°C (Enz Dil)  
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)

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**REAGENTS:** (continued)

E. Tyramine Oxidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.09 - 0.18 unit/ml of Tyramine Oxidase in cold Reagent D.)

**PROCEDURE:**

Prepare a reaction cocktail by adding Reagent C (POD) to Reagent A (Reaction Mix) for a final concentration of 5 - 15 units/ml of Peroxidase. Adjust to pH 7.5 at 37°C with 1 M KOH or 1 M HCl, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	1.00	1.00

Equilibrate to 37°C. Then add:

Reagent E (Enzyme Solution)	0.03	-----
Reagent D (Enz Dil)	-----	0.03

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

Reagent B (EtOH)	2.00	2.00
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Immediately mix by inversion and record the  $A_{480\text{nm}}$  for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{480\text{nm}} \text{ Test} - A_{480\text{nm}} \text{ Blank})(3.03)(\text{df})}{(17.17)(0.5)(10)(0.03)}$$

3.03 = Total volume (in milliliters) of stopped reaction

df = Dilution factor

17.17 = Millimolar extinction coefficient<sup>1</sup> of quinoneimine dye under the conditions of this assay

0.5 = Conversion factor derived from the fact that 2  $\mu\text{moles}$

of  $\text{H}_2\text{O}_2$  produce 1  $\mu\text{mole}$  of quinoneimine dye at 480nm

10 = Time (in minutes) of the assay as per the

Unit Definition

0.03 = Volume (in milliliter) of enzyme used in assay

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**CALCULATIONS:**

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

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

**UNIT DEFINITION:**

One unit will oxidize 1.0  $\mu$ mole of tyramine to p-hydroxyphenylacetaldehyde per minute at pH 7.5 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 1.03 ml reaction mix, the final concentrations are 20 mM potassium phosphate, 2.06 mM phenol, 1.44 mM 4-aminoantipyrine, 0.1 mM tyramine, 5 - 15 units peroxidase, and 0.0027 - 0.0054 unit tyramine oxidase.

**REFERENCES:**

Yamada, H., Hidehiko, K., and Uwajima, T. (1971) *Methods in Enzymology*, XVIIIB, 722-726

**NOTES:**

1. The extinction coefficient was determined by the supplier of the enzyme to Sigma.
2. This assay is based on the cited reference.
3. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.
4. 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.**