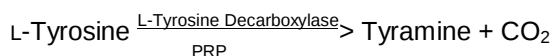


**SIGMA QUALITY CONTROL TEST
PROCEDURE****Enzymatic Assay of L-TYROSINE DECARBOXYLASE
(EC 4.1.1.25)****PRINCIPLE:**

Abbreviation used:
PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, pH 5.5

METHOD: Radiolabelled Stop Reaction

REAGENTS:

- A. 200 mM Sodium Phosphate Solution
(Prepare 50 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876.)
- B. 100 mM Citrate Phosphate Buffer, pH 5.5 at 37°C (Buffer)
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759. Adjust to pH 5.5 at 37°C with Reagent A.)
- C. McIlvaine's Buffer, pH 5.5 at 37°C (Buffer)
(Prepare using 50 ml of Reagent A and adjust to pH 5.5 at 37°C with Reagent B.)
- D. 2.65 mM L-Tyrosine Solution
(Prepare 10 ml in Reagent C using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat (70°C) may be required in order to solubilize.)
- E. 0.3 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 2 ml in Reagent C using Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255.)
- F. $^{14}\text{C}[\text{COOH}]$ L-Tyrosine
(Use $^{14}\text{C}[\text{COOH}]$ L-Tyrosine, 50 - 60 mCi/mmol, 50 $\mu\text{Ci/ml}$.)
- G. L-Tyrosine Reaction Cocktail (L-Tyr)
(Prepare by adding 0.05 ml of Reagent E to 2 ml of Reagent C.)

**Enzymatic Assay of L-TYROSINE DECARBOXYLASE
(EC 4.1.1.25)**

REAGENTS:

- H. 1 M Methylbenzethonium Hydroxide Solution (MBH)
(Use Benzethonium Hydroxide, approximately 1.0 M solution in Methanol, Sigma Prod. No. B2156.)
- I. Filter Paper
(1 cm x 2 cm strips of filter paper are prepared using Whatman 3 MM paper. Add 0.050 ml of Reagent G to each strip and allow to air dry. This step is to be carried out in a fume hood.)
- J. Vacutainer Tubes
(Use Vacutainer Blood Tubes, 13 x 75 mm, Becton Dickinson.)
- K. 25% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- L. L-Tyrosine Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.005-0.02 units/ml of L-Tyrosine Decarboxylase in cold Reagent C.)
- M. Scintillation Cocktail (Scint Fluid)
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)

PROCEDURE:

Radiolabelled CO₂ is trapped by adsorbing it on an organic solvent which is present on filter paper located above the reaction mixture in a vacutainer tube.

Pipette (in milliliters) the following reagents into suitable vacutainer tubes.

	<u>Test</u>	<u>Blank</u>	<u>PRP¹</u>
Reagent G (L-Tyr)	0.10	0.10	0.10
Reagent E (PRP)	0.10	0.10	-----
Reagent C (Buffer)	-----	-----	0.10

Equilibrate to 37°C. Then add:

Reagent L (Enz Soln)	0.10	-----	0.10
Reagent C (Buffer)	-----	0.10	-----

**Enzymatic Assay of L-TYROSINE DECARBOXYLASE
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PROCEDURE: (continued)

Immediately place Reagent H (Filter Paper) in the top of the vacutainer tube and seal the tubes by replacing the stopper. Incubate for exactly 10 or 20 minutes. Then add by injecting with a syringe and needle through the stopper. **(Do not remove stopper to add TCA.)**

	<u>Test</u>	<u>Blank</u>	<u>PRP¹</u>
Reagent K (TCA)	0.40	0.40	0.40

Mix by swirling. Allow to stand for 12-16 hours at room temperature to allow for all evolved CO₂ to be absorbed onto the filter paper.

Remove filter papers from vacutainer tubes and place into scintillation vials containing 7 ml of Reagent L (Scint). This should be performed in a hood.

Potential DPM (disintegration per minute) are prepared by pipetting 0.05 ml of Reagent F (L-Tyr) onto Reagent H (Filter Paper). Place into scintillation vial containing 7 ml of Reagent L (Scint Fluid).

CALCULATIONS:

$$\text{Potential DPM}/\mu\text{mole} = \frac{\text{DPM of potential}}{\text{Total } \mu\text{moles of L-Tyrosine}}$$

$$\text{Units/ml enzyme} = \frac{(\text{DPM Test} - \text{DPM Blank})(\text{df})}{(\text{T})(0.1)(\text{potential DPM}/\mu\text{mole})}$$

DPM = Disintegration per minute

df = Dilution factor

T = Time (in minutes) of assay as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will liberate 1.0 μmole of CO₂ for L-tyrosine per minute at pH 5.5 at 37°C.

**Enzymatic Assay of L-TYROSINE DECARBOXYLASE
(EC 4.1.1.25)**

FINAL ASSAY CONCENTRATION:

In a 0.30 ml reaction mix, the final concentrations are 100 mM citric acid³, 0.86 mM L- tyrosine, 0.1 mM pyridoxal 5-phosphate, and 0.0005-0.002 units L-tyrosine decarboxylase.

REFERENCE:

Sundaresan, P.R. and Coursin, D.B. (1970) *Methods in Enzymology*, XVIII, Part A, 509-512

NOTES:

1. The enzyme activity is also measured without any Reagent D(PRP) being present.
2. This assay is based on the cited reference.
3. This concentration is only an estimate since it is not known what exact volume of sodium phosphate is required to adjust the pH to 5.5 at 37°C.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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