Enzymatic Assay of TRYPTOPHANASE
(EC 4.1.99.1)

PRINCIPLE:

PRP + L-Tryptophan + H_2O $\xrightarrow{\text{Tryptophanase}}$ Indole + Pyruvate + NH_3

Abbreviation:
PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, pH 8.3, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 1000 mM Potassium Phosphate, Dibasic, Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Prod. No. P-5504.)

B. 1000 mM Potassium Phosphate, Monobasic, Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379.)

C. 1000 mM Potassium Phosphate Buffer, pH 8.3 at 37°C
(Prepare by equilibrating 100 ml of Reagent A to 37°C and adjusting the pH to 8.3 at 37°C with Reagent B.)

D. 0.81 mM Pyridoxal 5-Phosphate (PRP)
(Prepare 5 ml in deionized water using Pyridoxal 5-Phosphate, Prod. No. P-9255. **PREPARE FRESH.**)

E. 50 mM L-Tryptophan Solution, pH 10.8 at 37°C
(Prepare 25 ml in deionized water using L-Tryptophan, Prod. No. T-0254. Adjust to pH 10.8 at 37°C with 1 M NaOH.)

F. 100% (w/v) Trichloroacetic Acid (TCA)
(Use Trichloroacetic Acid, 6.1 N Solution, Stock No. 490-10.)

G. Toluene
(Use Toluene.)

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REAGENTS: (continued)

H. 95% Ethanol (Nondenatured)

I. 5% (w/v) p-Dimethylaminobenzaldehyde Solution (DMAB)
(Prepare 20 ml in Reagent H using p-Dimethylaminobenzaldehyde, Prod. No. D-2004. **PREPARE FRESH** and protect from light.)

J. 859 mM Hydrochloric Acid-Alcohol Reagent (Acid-Alcohol)
(Prepare by adding 8 ml of Reagent M to 100 ml of Reagent H.)

K. 0.43 mM Indole Standard Solution (Indole Std)
(Prepare 100 ml in deionized water using Indole, Prod. No. I-0750.)

L. Tryptophanase Enzyme Solution
(Immediately before use, prepare a solution containing 2 - 4 mg/ml in Reagent C.)

M. Hydrochloric Acid
(Use Hydrochloric Acid, Prod. No. H-7020.)

PROCEDURE:

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

	Test	Test Blank	Substrate Blank
Reagent C (Buffer)	0.20	0.20	0.40
Reagent D (PRP)	0.10	0.10	0.10
Reagent L (Enzyme Solution)		0.20	0.20
Deionized Water	1.30	1.50	1.30

Mix by inversion and equilibrate to 37°C. Then add:

Reagent E (L-Tryptophan Soln)	0.20	------	0.20

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

Reagent F (TCA)	0.20	0.20	0.20

PROCEDURE: (continued)

Mix by inversion. Add 2.0 ml of Reagent G (Toluene) to the Test, Test Blank, and Substrate Blank. Mix vigorously to phase extract the liberated indole. Allow the phases to separate and then pipette (in milliliters) the following reagents into suitable containers:

	Test	Test Blank	Substrate Blank
Aliquot from Toluene Layer	0.20	0.20	0.20
Reagent I (DMAB)	1.00	1.00	1.00
Reagent J (Acid-Alcohol)	8.80	8.80	8.80

Mix by inversion and allow to equilibrate at 25°C for 10 minutes. Transfer the solutions to suitable cuvettes and record the A_{540nm} for the Test, Test Blank, and Substrate Blank using a suitably thermostatted spectrophotometer.

COLORIMETRIC ASSAY:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Standard Blank
Reagent C (Buffer)	0.20	0.20	0.20	0.20
Reagent K (Indole Std)	0.10	0.20	0.30	------
Deionized Water	1.70	1.60	1.50	1.80

Mix by inversion and then add:

Reagent F (TCA)	0.20	0.20	0.20	0.20

Mix by inversion and add 2.0 ml of Reagent G (Toluene) to each container. Mix vigorously to phase extract the indole. Allow the phases to separate and then pipette (in milliliters) the following reagents into suitable containers.

Aliquot from Toluene Layer	0.20	0.20	0.20	0.20
Reagent I (DMAB)	1.00	1.00	1.00	1.00

Reagent J (Acid-Alcohol)	8.80	8.80	8.80	8.80

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COLORIMETERIC ASSAY: (continued)

Mix by inversion and allow to equilibrate at 25°C for 10 minutes. Transfer the solutions to suitable cuvettes and record the A_{540nm} for the Standards and Standard Blank, using a suitably thermostatted spectrophotometer.

CALCULATION:

Standard Curve:

r A_{540nm} Standard = A_{540nm} Standard - A_{540nm} Standard Blank

Prepare a standard curve by plotting the ?A_{540nm} of the Standard vs micrograms of indole.

CALCULATIONS: (continued)

Sample Determination:

r A_{540nm} = A_{540nm} Test - (A_{540nm} Test Blank + A_{540nm} Substrate Blank)

Determine the total micrograms of indole liberated using the Standard curve.

$$\text{Units/mg enzyme} = \frac{(\mu g \text{ indole released})}{(\text{mg enzyme/RM})}$$

RM = Reaction Mix

UNIT DEFINITION:

One mg will release 15 - 40 µg of indole from L-tryptophan in 10 minutes at pH 8.3 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 200 mM potassium phosphate, 0.041 mM pyridoxal 5-phosphate,
5 mM tryptophan, and 0.4 - 0.8 mg tryptophanase.

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NOTES:

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.