

## Enzymatic Assay of APOTRYPTOPHANASE

### PRINCIPLE:

PRP + L-Tryptophan + H<sub>2</sub>O ~~Apotryptophanase~~ > Indole + Pyruvate + NH<sub>3</sub>

Abbreviation:

PRP = Pyridoxal 5-Phosphate

**CONDITIONS:** T = 37°C, pH = 8.3, A<sub>540nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

### REAGENTS:

- A. 1 M Potassium Phosphate, Dibasic, Solution  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- B. 1 M Potassium Phosphate, Monobasic, Solution  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- C. 1 M 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, Sigma Prod. No. P-9255. **PREPARE FRESH.**)
- E. 50 mM L-Tryptophan Solution, pH 10.2 at 37°C  
(Prepare 25 ml in deionized water using L-Tryptophan, Sigma Prod. No. T-0254. Adjust to pH 10.2 at 37°C with 1 M NaOH.)
- F. 100% (w/v) Trichloroacetic Acid (TCA)  
(Use Trichloroacetic Acid, 6.1 N Solution, approximately 100%, Sigma Stock No. 490-10.)
- G. Toluene  
(Use Toluene, Sigma Stock No. 27,037-7.)

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**REAGENTS:** (continued)

- H. 95% (v/v) Ethanol  
(Prepare 125 ml in deionized water using, 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)
- I. 5% (w/v) p-Dimethylaminobenzaldehyde Solution (DMAB)  
(Prepare 20 ml in Reagent H using p-Dimethylaminobenzaldehyde, Sigma Prod. No. D-2004. **PREPARE FRESH** and protect from light.)
- J. Hydrochloric Acid  
(Use Hydrochloric Acid, Sigma Prod. No. H-7020.)
- K. 859 mM Hydrochloric Acid-Alcohol Reagent (Acid-Alcohol)  
(Prepare by adding 8 ml of Reagent J to 100 ml of Reagent H.)
- L. 0.43 mM Indole Standard Solution (Indole Std)  
(Prepare 100 ml in deionized water using Indole, Sigma Prod. No. I-0750.)
- M. Apotryptophanase Enzyme Solution  
(Immediately before use, prepare a solution containing 2 - 4 mg/ml in Reagent C.)

**PROCEDURE:**

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

		Test	
	<u>Test</u>	<u>Blank</u>	<u>Substrate Blank</u>
Reagent C (Buffer)	0.20	0.20	0.40
Reagent D (PRP)	0.10	0.10	0.10
Reagent M (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
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Immediately mix by inversion and incubate at 37°C for

exactly 10 minutes. Then add:

Reagent F (TCA)	0.20	0.20	0.20
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### 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. The toluene layer will be the upper layer. Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>	<u>Substrate Blank</u>
Aliquot from Toluene Layer	0.20	0.20	0.20
Reagent I (DMAB)	1.00	1.00	1.00
Reagent K (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:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Standard Blank</u>
Reagent C (Buffer)	0.20	0.20	0.20	0.20
Reagent L (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
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Mix by inversion and add 2.0 ml of Reagent G (Toluene) to each container. Mix vigorously to phase extract the liberated indole. The toluene layer will be the upper layer. 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
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Reagent I (DMAB)	1.00	1.00	1.00	1.00
Reagent K (Acid-Alcohol)	8.80	8.80	8.80	8.80

## Enzymatic Assay of APOTRYPTOPHANASE

### 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  $A_{540\text{nm}}$  for the Standards and Standard Blank, using a suitably thermostatted spectrophotometer.

### CALCULATION:

Standard Curve:

$$r A_{540\text{nm}} \text{ Standard} = A_{540\text{nm}} \text{ Standard} - A_{540\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting the  $r A_{540\text{nm}}$  of the Standard vs micrograms of indole.

Sample Determination:

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

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

$$\text{Units/ml enzyme} = \frac{(\mu\text{g indole released})(\text{df})}{(0.2)}$$

df = Dilution factor

0.2 = Volume (in milliliter) of enzyme used

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

### ACTIVITY:

One mg will release 75-150  $\mu\text{g}$  of indole from L-tryptophan in 10 minutes at pH 8.3 at 37°C in the presence of  $4 \times 10^{-2}$  mM pyridoxal 5'-phosphate.

### 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 apotryptophanase.

## Enzymatic Assay of APOTRYPTOPHANASE

### REFERENCE:

Gunsalus, I.C., Galeener, C.C., and Stamer, J.R. (1955)  
*Methods in Enzymology*, Volume II, 238-242

### NOTES:

1. This assay is based on the cited reference.
2. 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.**