

**Enzymatic Assay of ASPARTATE TRANSCARBAMYLASE  
(EC 2.1.3.2)**

**PRINCIPLE:**

Carbamyl Phosphate + L-Aspartate  $\xrightarrow{\text{Aspartate Transcarbamylase}}$  N-Carbamyl-Aspartate  
+ Orthophosphate

**CONDITIONS:** T = 37°C, pH = 8.5, A<sub>466nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 1 M Tris HCl Buffer, pH 8.5 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 37°C with 1 M HCl.)
- B. 100 mM Carbamyl Phosphate Solution<sup>1</sup> (Carbam Phos)  
(Prepare 1 ml in cold (0 - 5°C) deionized water using Carbamyl Phosphate, Dilithium Salt, Sigma Prod. No. C-5625. Store at 0 - 5°C. **PREPARE FRESH.**)
- C. 200 mM L-Aspartic Acid Solution (L-Asp)  
(Prepare 5 ml in deionized water using L-Aspartic Acid, Free Acid, Sigma Prod. No. A-9256. Adjust to pH 8.0 - 8.5 at 37°C with 1 M KOH. **PREPARE FRESH.**)
- D. 5% (w/v) Trichloroacetic Acid Solution (TCA)  
(Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- E. 18 N Sulfuric Acid Solution (H<sub>2</sub>SO<sub>4</sub>)  
(Prepare 25 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526. Store at 0 - 5°C.)
- F. 0.5% (w/v) Antipyrine Solution (AP)  
(Prepare 25 ml in Reagent E using Antipyrine, Sigma Prod. No. A-5882. Store at 0 - 5°C.)
- G. 5% (v/v) Glacial Acetic Acid Solution (Acet Acid)  
(Prepare 50 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283. Store at 0 - 5°C.)

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**REAGENTS:** (continued)

- H. 15.8 mM 2,3-Butanedione Monoxime Solution  
(Prepare 15 ml in Reagent G using 2,3-Butanedione Monoxime, Sigma Prod. No. B-0753. Store at 0 - 5°C.)
- I. Color Reagent solution (CRS)  
(Immediately before use, prepare 30 ml by cautiously mixing 20 ml of Reagent F with 10 ml of Reagent H.)
- J. 10 mM N-Carbamyl-DL-Aspartate Standard Solution (Std Soln)  
(Prepare 10 ml in Reagent A using N-Carbamyl-DL-Aspartic Acid, Sigma Prod. No. C-4250. Adjust to pH 7.0 - 9.0 with 10 N KOH. **PREPARE FRESH.**)
- K. Aspartate Transcarbamyase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Aspartate Transcarbamyase in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.10	0.10
Reagent B (Carbam Phos)		0.10
		0.10
Reagent C (L-Asp)	0.50	0.50
Deionized Water	0.20	0.20

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

Reagent K (Enzyme Solution)	0.10	-----
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Mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent D (TCA)	2.00	2.00
Reagent K (Enzyme Solution)	-----	0.10

Mix by swirling.

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**COLOR DEVELOPMENT:**

Standard Curve:

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>Std 4</u>	<u>Std Blank</u>
Reagent J (Std Soln)	0.25	0.50	0.75	1.00	-----
Deionized Water	0.75	0.50	0.25	-----	1.00
Reagent I (CRS)	2.00	2.00	2.00	2.00	2.00

Mix by swirling and heat at 60°C in a water bath for 120 minutes. Remove from the water bath and cool to 0 - 4°C in an ice bath.

Sample:

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

	<u>Test</u>	<u>Blank</u>
Test Aliquot	1.00	-----
Blank Aliquot	-----	1.00
Reagent I (CRS)	2.00	2.00

Mix by swirling and heat at 60°C in a water bath for 120 minutes. Remove from the water bath and cool to 0 - 4°C in an ice bath.

Transfer the solutions to suitable cuvettes and record the  $A_{466\text{nm}}$  for the Test, Blank, Standards, and Standard Blank using a suitable spectrophotometer.

**CALCULATIONS:**

Standard Curve:

$$r A_{466\text{nm}} \text{ Standard} = A_{466\text{nm}} \text{ Standard} - A_{466\text{nm}} \text{ Standard Blank}$$

Plot the  $r A_{466\text{nm}}$  Standard versus  $\mu\text{moles}$  of N-Carbamyl-Aspartate.

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**CALCULATIONS:** (continued)

Sample Determination:

$$r A_{466\text{nm}} \text{ Sample} = A_{466\text{nm}} \text{ Test} - A_{466\text{nm}} \text{ Test Blank}$$

Determine the  $\mu\text{moles}$  of N-Carbamyl-Aspartate produced using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Carbamyl-Aspartate produced})(3)(\text{df})}{(30)(0.1)}$$

3 = Total volume (in milliliters) of stopped reaction

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

**UNIT DEFINITION:**

One unit will catalyze the formation of 1.0  $\mu\text{mole}$  of N-carbamyl-L-aspartate per minute at pH 8.5 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 1.00 ml reaction mix, the final concentrations are 100 mM Tris, 10 mM carbamyl phosphate, 100 mM L-aspartic acid, and 0.01 - 0.02 unit aspartate transcarbamylase.

**REFERENCE:**

Prescott, L.M. and Jones, M.E. (1969) *Analytical Biochemistry* **32**, 408-419

**NOTES:**

1. Carbamyl Phosphate is very unstable in solution and therefore should be made fresh before each assay is performed. Material should be weighed in a dry atmosphere.

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**NOTES:** (continued)

2. This assay is based on the cited reference.
3. 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.**