Enzymatic Assay of ORNITHINE CARBAMYL TRANSFERASE (EC 2.1.3.3)

PRINCIPLE:

Carbamyl phosphate + $_{L}$-Ornithine $\xrightarrow{OCT}$ $_{L}$-Citrulline + P_i

Abbreviations:
P_i = Inorganic Phosphate
OCT = Ornithine Carbamyl Transferase

CONDITIONS: T = 37°C, pH = 8.5, A_{490nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 10 mM Tris HCl, 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. 50 mM $_{L}$-Ornithine Solution ($_{L}$-Orn)
(Prepare 10 ml in deionized water using $_{L}$-Ornithine Hydrochloride, Sigma Prod. No. O-2375. Adjust to pH 7.5 at 25°C with solid Sodium Bicarbonate, Sigma Prod. No. S-8875. **PREPARE FRESH**.)

C. 50 mM Carbamyl Phosphate Solution (CP)
(Prepare 5 ml in cold deionized water using Carbamyl Phosphate, Dilithium Salt, Sigma Prod. No. C-5625. Keep in an ice bath while using. **PREPARE FRESH**.)

D. 6.25% (w/v) Trichloroacetic Acid Solution (TCA)
(Use Trichloroacetic Acid, 0.38 N Solution, approximately 6.25% (w/v), Sigma Stock No. 331-7.)

E. Redox Buffer
(Prepare by dissolving 0.9 g of Ferric Ammonium Sulfate, Dodecahydrate, Sigma Prod. No. F-3629, and 1.1 g Ferrous Ammonium Sulfate, Hexahydrate, Sigma Prod. No. F-2262, in 10 ml of Reagent F.)

REAGENTS: (continued)

F. 1 M Sulfuric Acid Solution (H_2SO_4)
(Prepare 50 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526.)

G. 7.4 M Phosphoric Acid with 3.0 M Sulfuric Acid Solution (Acid)
(Prepare 60 ml in deionized water using Phosphoric Acid, Sigma Prod. No. P-6560 and Sulfuric Acid, Sigma Prod. No. S-1526.)

H. 75 mM 2,3-Butanedione Monoxime Solution (BuD)
(Prepare 25 ml in deionized water using 2,3-Butanedione Monoxime, Sigma Prod. No. B-0753.)

I. 10 mM Citrulline Stock Solution (Citrul)
(Prepare 100 ml in deionized water using L-Citrulline, Sigma Prod. No. C-7629.)

J. 1 mM Citrulline Standard Solution (STD)
(Prepare by adding 1 ml of Reagent I to 9 ml deionized water. **PREPARE FRESH**.)

K. Ornithine Carbamyl Transferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1 unit/ml in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.30	2.30
Reagent B (L-Orn)	0.30	0.30
Reagent C (CP)	0.30	0.30

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

Reagent K (Enzyme Solution)	0.10	------

Mix by inversion and incubate at 37°C for exactly 15 minutes. Then add:

Reagent D (TCA)	3.00	3.00
Reagent K (Enzyme Solution)	------	0.10

Mix by inversion.

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COLORIMETRIC DETERMINATION:

Pipette (in milliliters) the following into suitable tubes (Mix by swirling after each addition):

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Deionized Water	----	-----	0.90	0.80	0.60	0.40	0.20	1.00
Test	1.00	-----	-----	-----	-----	-----	-----	-----
Test Blank	----	1.00	-----	-----	-----	-----	-----	-----
Reagent J (STD)	----	-----	0.10	0.20	0.40	0.60	0.80	-----
Reagent E (Redox)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Reagent G (Acid)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Reagent H (BuD)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Mix by swirling. Cover all tubes with a glass marble. Transfer tubes to a boiling water bath and incubate for 20 minutes.

Transfer tubes to a cold water bath and cool to room temperature. **PROTECT FROM LIGHT.**

Then add:

Deionized Water	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00

Mix by swirling, then transfer the solutions to suitable cuvettes and record the absorbance at A_{490nm} for Test, Blank and Standards using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$r\ A_{490nm}$ Standard = A_{490nm} Standard - A_{490nm} Standard Blank

Plot the $r\ A_{490nm}$ Standard vs µmoles of citrulline.

Sample Determination:

$r\ A_{490nm}$ Sample = A_{490nm} Test - A_{490nm} Test Blank

Determine the µmoles of citrulline liberated using the Standard curve.

CALCULATIONS: (continued)

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of citrulline liberated})\ (6)\ (df)}{(15)\ (0.1)\ (1)}$$

6 = Volume (in milliliters) of assay
df = Dilution factor
15 = Time of assay (in minutes) as per the Unit Definition
0.1 = Volume (in milliliters) of enzyme used
1 = Volume (in milliliters) of assay used in Colorimetric Determination

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

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

UNIT DEFINITION:

One unit will form 1.0 µmole of citrulline from ornithine and carbamyl phosphate per minute at pH 8.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 8 mM Tris, 5 mM L-ornithine, 5 mM carbamyl phosphate and 0.05 - 0.1 unit ornithine carbamyl transferase.

REFERENCE:

Marshall, M. and Cohen, P.P. (1972) *Journal of Biological Chemistry* **247**, 1641-1653.

NOTES:

1. If the solutions are not clear, they may be clarified by filtration or by centrifugation.

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.