



## SIGMA QUALITY CONTROL TEST PROCEDURE

## Product Information

### Enzymatic Assay of AMINOACYL-tRNA SYNTHETASE (EC 6.1.1)

#### PRINCIPLE:

tRNA +  $^{14}\text{C}$  Arginine + ATP  $\rightarrow$  tRNA -  $^{14}\text{C}$  Arginine + AMP + Pyrophosphate

**CONDITIONS:** T = 37°C, pH = 7.6

**METHOD:** Radiolabelled Stop Reaction

#### REAGENTS:

- A. 1 M Tris HCl Buffer with 50 mM Magnesium Chloride, 500 mM Potassium Chloride, 5 mM Ethylenediaminetetraacetic Acid, and 25 mM Adenosine 5'-Triphosphate, pH 7.6 at 37°C (Reaction Mix Buffer)  
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, Potassium Chloride, Sigma Prod. No. P-4504, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS, and Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. Add all reagents except Adenosine 5'-Triphosphate, adjust the pH to 7.6 and place on ice. Then add the appropriate amount of Adenosine 5'-Triphosphate.)
- B.  $^{14}\text{C}$  Arginine Solution ( $^{14}\text{C}$  Arg)  
(Use  $^{14}\text{C}$  L-Arginine, 300 mCi/mmol, 50  $\mu\text{Ci/ml}$ .)
- C. Transfer RNA Solution (tRNA)  
(Prepare 1 ml in deionized water containing 160  $A_{260}$  units/ml.)<sup>1</sup>
- D. 10 mM Tris HCl Buffer, with 50% (v/v) Glycerol, 10 mM Magnesium Chloride, 10 mM Potassium Chloride, and 30 mM 2-Mercaptoethanol, pH 7.2 at 25°C (Enz Dil)  
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Glycerol, Sigma Prod. No. G-7893, Potassium Chloride, Sigma Prod. No. P-4504, 2-Mercaptoethanol, Sigma Prod. No. M-6250, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

**Enzymatic Assay of AMINOACYL-tRNA SYNTHETASE  
(EC 6.1.1)**

**REAGENTS:** (continued)

- E. Aminoacyl-tRNA Synthetase Enzyme Solution<sup>2</sup>  
(Immediately before use, prepare a solution containing 1 - 4 mg/ml (based on protein) of Aminoacyl-tRNA Synthetase in ice cold Reagent D.)
- F. 10% (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. Store on ice.)
- G. 5% (w/v) Trichloroacetic Acid Solution (Wash Solution)  
(Prepare 5 ml in deionized water using Reagent F.)
- H. Methylene Cellosolve  
(Prepare by adding equal volumes of Ethylene Glycol Monoethyl Ether, Sigma Prod. No. E-2632, and Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378.)
- I. Scintillation Cocktail  
(Use Sigma-Fluor Universal LSC cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)

**PROCEDURE:**

Pipette (in milliliters) the following reagents into suitable one dram vials:

	<u>Test</u>	<u>Blank</u>
Deionized Water	0.370	0.370
Reagent A (Reaction Mix Buffer)	0.050	0.050
Reagent C (tRNA)	0.050	0.050
Reagent B ( <sup>14</sup> C Arg)	0.020	0.020

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

Reagent E (Enzyme Solution)	0.01	-----
Reagent D (Enz Dil)	-----	0.01

Immediately mix by swirling and incubate at 37°C for exactly 10 minutes. Then remove three 0.050 ml aliquots from both the Test and Blank and place into suitable containers containing 0.2 ml of ice cold Reagent F (TCA). Allow the samples to stand for 5 - 10 minutes on ice.

Filter the solutions through 0.45 µm Millipore HA Type filters. Wash the filters 3 times with 0.100 ml each of Reagent G (Wash Solution).

## Enzymatic Assay of AMINOACYL-tRNA SYNTHETASE (EC 6.1.1)

### PROCEDURE: (continued)

Allow the filters to air dry or dry under a heat lamp and transfer them to 2 dram scintillation vials. Dissolve the filters in 2 ml of Reagent H (Methylethyl Cellosolve), then add 5 ml of Reagent I (Scintillation Cocktail). Only use filters that are soluble in Reagent H (Methylethyl Cellosolve). Count the radioactivity in a suitable scintillation counter.

Potential DPM (disintegrations per minute) are prepared by pipetting 0.02 ml of Reagent B ( $^{14}\text{C}$  Arg) into a scintillation vial with 2 ml of Reagent H (Methylethyl Cellosolve) and 5 ml of Reagent I (Scintillation Cocktail).

### CALCULATIONS:

$$\text{Potential DPM/pmole} = \frac{\text{DPM of potential}}{\text{Total pmoles of L-Arginine}}$$

$$\text{Units/ml enzyme} = \frac{(\text{DPM Test} - \text{DPM Blank})(\text{df})(0.5)}{(0.01)(\text{Potential DPM/pmole})(0.05)}$$

DPM = Disintegrations per minute

df = Dilution factor

0.5 = Volume (in milliliter) of assay

0.01 = Volume (in milliliter) of enzyme used

0.05 = Volume (in milliliter) of reaction mixture which is added to the scintillation cocktail

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

### UNIT DEFINITION:

One unit will activate and attach 1.0 picomole ( $10^{-12}$  mole) of labeled amino acid to tRNA in 10 minutes at pH 7.6 at 37°C (amino acid used: L-arginine).

### FINAL ASSAY CONCENTRATION:

In a 0.50 ml reaction mix, the final concentrations are 100 mM Tris, 5 mM magnesium chloride, 50 mM potassium chloride, 0.5 mM ethylenediaminetetraacetic acid, 2.5 mM adenosine 5'-triphosphate, 8 units t-RNA, 6.67  $\mu\text{M}$  arginine, 1.0% (w/v) glycerol, 0.6 mM 2-mercaptoethanol, and 0.01 - 0.04 mg aminoacyl tRNA synthetase.

**Enzymatic Assay of AMINOACYL-tRNA SYNTHETASE  
(EC 6.1.1)**

**REFERENCE:**

Nishimura, S., Harada, F., Narushima, U., and Seno, T. (1967) *Biochimica Et Biophysica Acta* **142**, 133-148

**NOTES:**

1. The type of t-RNA used in the assay depends on the source of the t-RNA Synthetase. For Aminoacyl-tRNA Synthetase, Crude from Baker's Yeast, Sigma Prod. No. A-6302, use Ribonucleic Acid, Transfer, Type X from Baker's Yeast, Sigma Prod. No. R-9001; for Aminoacyl-tRNA Synthetase, Crude from Bovine Liver, Sigma Prod. No. A-3518, Aminoacyl-tRNA Synthetase from Rabbit Liver, use Sigma Prod. No. A-9018, and for Aminoacyl-tRNA Synthetase, Crude from E. Coli, Sigma Prod. No. A-3646, use Ribonucleic acid, Transfer, Type XXI from Escherichia coli, Sigma Prod. No. R-4251.)
2. This enzyme is extremely unstable and should not be exposed to room temperature for more than a few minutes. It should also not be exposed to repeated freeze-thaw cycles.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.**