

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

**PRINCIPLE:**

tRNA + <sup>14</sup>C Arginine + ATP → tRNA - <sup>14</sup>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. <sup>14</sup>C Arginine Solution (<sup>14</sup>C Arg)  
(Use <sup>14</sup>C L-Arginine, 300 mCi/mmol, 50 μCi/ml.)
- C. Transfer RNA Solution (tRNA)  
(Prepare 1 ml in deionized water containing 160 A<sub>260</sub> 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.)

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**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).

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**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 (<sup>14</sup>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  $\mu$ M arginine, 1.0% (w/v) glycerol, 0.6 mM 2-mercaptoethanol, and 0.01 - 0.04 mg aminoacyl tRNA synthetase.

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**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.

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