Enzymatic Assay of AMINOACYL-tRNA SYNTHETASE
(EC 6.1.1)

PRINCIPLE:

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

CONDITIONS: $T = 37^\circ C$, 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)

(Begin with 25 ml in deionized water using Trizma Base, Magnesium Chloride, Hexahydrate, Potassium Chloride, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, and Adenosine 5'-Triphosphate. 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. $^{14}$C Arginine Solution ($^{14}$C Arg)

(Use $^{14}$C L-Arginine, 300 mCi/mmol, 50 µCi/ml.)

C. Transfer RNA Solution (tRNA)

(Prepare 1 ml in deionized water containing 160 A$_{260}$ units/ml.)

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, Glycerol, Potassium Chloride, 2-Mercaptoethanol, and Magnesium Chloride, Hexahydrate.)
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REAGENTS: (continued)

E. Aminoacyl-tRNA Synthetase Enzyme Solution
(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), Store on ice.)

G. 5% (w/v) Trichloroacetic Acid Solution (Wash Solution)
(Prepare 5 ml in deionized water using Reagent F.)

H. Methylethyl Cellosolve
(Prepare by adding equal volumes of Ethylene Glycol Monoethyl Ether, and Ethylene Glycol Monomethyl Ether.)

I. Scintillation Cocktail
(Use Sigma-Fluor Universal LSC cocktail for Aqueous Samples.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.370</td>
<td>0.370</td>
</tr>
<tr>
<td>Reagent A (Reaction Mix Buffer)</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Reagent C (tRNA)</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Reagent B (14C Arg)</td>
<td>0.020</td>
<td>0.020</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.01</td>
<td>------</td>
</tr>
<tr>
<td>Reagent D (Enz Dil)</td>
<td>------</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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).
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 ($^{14}$C Arg) into a scintillation vial with 2 ml of Reagent H (Methylethyl Cellosolve) and 5 ml of Reagent I (Scintillation Cocktail).

CALCULATIONS:

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

\[ \frac{(\text{DPM Test} - \text{DPM Blank})(\text{df})(0.5)}{(\text{DPM Test} - \text{DPM Blank})(\text{df})(0.5)} \]

Units/ml enzyme = \[ \frac{\text{Units/ml enzyme}}{\text{mg protein/ml enzyme}} \]

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

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 µM 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:

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, use Ribonucleic Acid, Transfer, Type X from Baker's Yeast; for Aminoacyl-tRNA Synthetase, Crude from Bovine Liver, Aminoacyl-tRNA Synthetase from Rabbit Liver, use and for Aminoacyl-tRNA Synthetase, Crude from E. Coli, use Ribonucleic acid, Transfer, Type XXI from Escherichia coli.)

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.

This procedure is for informational purposes.