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)  
(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. $^{14}$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)  
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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), 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. Methylethyl 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</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 ((^{14})C 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></th>
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<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 \(\mu\)m 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 (14C Arg) into a scintillation vial with 2 ml of Reagent H (Methylethyl Cellosolve) and 5 ml of Reagent I (Scintillation Cocktail).

CALCULATIONS:

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

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

\[\text{DPM} = \text{Disintegrations per minute}\]
\[\text{df} = \text{Dilution factor}\]
\[0.5 = \text{Volume (in milliliter) of assay}\]
\[0.01 = \text{Volume (in milliliter) of enzyme used}\]
\[0.05 = \text{Volume (in milliliter) of reaction mixture which is added to the scintillation cocktail}\]

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

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 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:


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.*