Enzyme Assay of ARYLAMINE ACETYLTRANSFERASE
(EC 2.3.1.5)

PRINCIPLE:

\[ \text{Acetyl-CoA} + \text{Dye} \xrightarrow{\text{AAT}} \text{CoASH} + \text{Acetylated Dye} \]

Abbreviations used:

- Acetyl-CoA = Acetyl Coenzyme A
- Dye = p-Nitroaniline
- AAT = Arylamine Acetyltransferase
- CoASH = Coenzyme A

CONDITIONS:

- \( T = 25^\circ C, \ \text{pH} = 8.0, \ A_{400nm}, \ \text{Light path} = 1 \text{ cm} \)

METHODS: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 8.0 at 25°C
   (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.0 at 25°C with 1 M KOH.)

B. 100 mM \( \beta \)-Mercaptoethanol Solution (\( \beta \)-ME)
   (Prepare 5 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

C. 30 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
   (Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 1.5 mM p-Nitroaniline Solution (Dye)
   (Prepare 5 ml in deionized water using p-Nitroaniline, Sigma Prod. No. N-2128.)

E. 6.0 mM Acetyl Coenzyme A Solution (Acetyl CoA)
   (Prepare 1 ml in deionized water using Acetyl Coenzyme A (C2:0), Sodium Salt, Sigma Prod. No. A-2056. PREPARE FRESH.)
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REAGENTS:  (continued)

F. Arylamine Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing
100 mg/ml of Arylamine Acetyltransferase in cold
Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.50</td>
</tr>
<tr>
<td>Reagent B (ß-ME)</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent C (EDTA)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Dye)</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the
A_{400nm} until constant (approx. 4 minutes) using a suitably
thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Acetyl CoA)</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in
A_{400nm} for approximately 5 minutes. Obtain the \( r A_{400nm}/\text{minute} \)
using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r \text{ A}_{400\text{nm}} \text{ Test} - r \text{ A}_{400\text{nm}} \text{ Blank})(1000)(3)(df)}{(11.19)(1)}
\]

1000 = Conversion from µmoles to nanomoles
3 = Volume (in milliliters) of assay
df = Dilution factor
11.19 = Millimolar extinction coefficient of acetylated
dye
1 = Volume (in milliliters) of enzyme used

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]
Enzyme Assay of ARYLAMINE ACETYLTRANSFERASE (EC 2.3.15)

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will acetylate 1.0 nanomole of \( p \)-nitroaniline per minute at pH 8 at 25°C.

FINAL CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM potassium phosphate, 5 mM \( \beta \)-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 0.1 mM \( p \)-nitroanilne, 0.1 mM acetyl coenzyme A and 100 mg of arylamine acetyltransferase.

REAGENTS:


NOTES:

1. This assay is based on the cited reference.

2. 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.