Enzymatic Assay of ARYL ACYLMIDASE  
(EC 3.5.1.13)

PRINCIPLE:
N-Acetyl-p-Aminophenol + H₂O → p-Aminophenol + Acetate

CONDITIONS:  T = 37°C, pH = 9.0, A₆₁₅nm, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 9.0 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base. Adjust to pH 9.0 at 37°C with 1 M HCl.)

B. 100 mM N-Acetyl-p-Aminophenol Solution (NAAP)  
(Prepare 50 ml in Reagent A using 4-Acetamidophenol.)

C. 5.0 mM p-Aminophenol Standard Solution (Std)  
(Prepare by dissolving 54.56 mg of p-Aminophenol, in 80 ml of deionized water. Adjust the pH to 11.0 at 25°C with 0.1 M NaOH. The solution should be clear and purple at this point. Adjust the pH back to 9.0 using 0.1 M HCl. The color of the solution should turn brown. Quantitatively transfer to a 100 ml volumetric flask and dilute to 100 ml with deionized water. Protect the solution from light and use immediately after preparation.)

D. 270 mM Ammonium Hydroxide Solution (NH₄OH)  
(Prepare 100 ml in deionized water using Ammonium Hydroxide.)

E. 1.28 mM Cupric Sulfate Solution (CuSO₄)  
(Prepare 100 ml in Reagent D using Cupric Sulfate, Pentahydrate.)

F. 0.41% (v/v) o-Cresol Solution  
(Prepare 48.2 ml by adding 0.2 ml of o-Cresol to 48 ml of deionized water. PREPARE FRESH.)
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REAGENTS: (continued)

G. Color Reagent Solution (CRS)
(Prepare 54.2 ml by adding 48.2 ml of Reagent F to 6 ml of Reagent E. Mix well. Prepare fresh and use within 30 minutes.)

H. Aryl Acylamidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.13 - 0.25 unit/ml of Aryl Acylamidase 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>-----</td>
</tr>
<tr>
<td>Reagent B (NAAP)</td>
<td>2.90</td>
</tr>
</tbody>
</table>

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

| Reagent H (Enzyme Solution) | 0.10 | ----- |

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes.

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

| Reagent G (CRS) | Test 2.50 | Blank 2.50 |
| Test Mixture | 1.00 | ----- |
| Blank Mixture | ----- | 1.00 |

Mix by swirling and incubate at room temperature for exactly 5 minutes. Transfer the solutions to suitable cuvettes and record the A615nm for the Test and Blank using a suitable spectrophotometer.

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Std)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>0.02</td>
<td>---</td>
</tr>
<tr>
<td>Reagent B (NAAP)</td>
<td>2.90</td>
<td>2.90</td>
<td>2.90</td>
<td>2.90</td>
<td>2.90</td>
</tr>
</tbody>
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**PROCEDURE:** (continued)

Mix by swirling and incubate at 37°C for exactly 10 minutes.

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

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<tr>
<th></th>
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<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (CRS)</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Std 1</td>
<td>1.00</td>
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</tr>
<tr>
<td>Std 2</td>
<td>---</td>
<td>1.00</td>
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<td>1.00</td>
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<td>1.00</td>
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<tr>
<td>Blank</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at room temperature for exactly 5 minutes. Transfer the solutions to suitable cuvettes and record the $A_{615nm}$ for the Standards and Standard Blank using a suitable spectrophotometer.

**CALCULATIONS:**

Standard Curve:

$r \quad A_{615nm} \text{ Standard} = A_{615nm} \text{ Standard} - A_{615nm} \text{ Standard Blank}$

Plot the $r A_{615nm} \text{ Standard}$ vs µmoles of p-Aminophenol.

Sample Determination:

$r \quad A_{615nm} \text{ Sample} = A_{615nm} \text{ Test} - A_{615nm} \text{ Test Blank}$

Determine the µmoles of p-Aminophenol liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{µmoles of p-Aminophenol released})(df)}{(10)(0.1)}$$

$\text{df} = \text{Dilution factor}$
$10 = \text{Time (in minutes) of assay as per the Unit Definition}$
$0.1 = \text{Volume (in milliliter) of enzyme used in the assay}$

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$
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CALCULATIONS:  (continued)

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of N-acetyl-p-aminophenol (acetaminophen) to p-aminophenol per minute at pH 9.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 100 mM Tris, 97 mM N-acetyl-p-aminophenol, and 0.0125 - 0.025 unit aryl acylamidase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

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