Arylacylamidase catalyzes the conversion of p-nitroacetanilide to p-nitroaniline, which absorbs strongly at 405 nm:

\[ \text{p-Nitroacetanilide} + \text{H}_2\text{O} \rightarrow \text{p-Nitroaniline} + \text{Acetic acid} \]

Arylacylamidase also catalyzes the conversion of acetaminophen to p-aminophenol:

\[ \text{Acetaminophen} + \text{H}_2\text{O} \rightarrow \text{p-Aminophenol} + \text{Acetic acid} \]

In one method, p-aminophenol reacts with o-cresol in the presence of periodate to form an indophenol, which absorbs strongly at 615 nm.
Specifications

Form
White, off-white, or yellow lyophilized powder.

Activity
≥15 U/mg powder.

Unit
One unit is defined as the amount of enzyme which catalyzes the conversion of 1 µmole of p-nitroacetanilide to p-nitroaniline at 30°C, pH 8.5 under the conditions given in the assay procedure.

Properties

Solubility
Arylacylamidase is soluble in water and buffers.

Effect of Buffers
Arylacylamidase is stable in pH 8.5 Tris and Phosphate buffers with a molarity of 20 mM to 100mM. The following chart shows the percent activity obtained when assayed in various Tris and Phosphate buffers.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Tris</th>
<th>Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mM, pH 8.5</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>50 mM, pH 8.5</td>
<td>100%</td>
<td>97%</td>
</tr>
<tr>
<td>100 mM, pH 8.5</td>
<td>94%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Optimum pH and Temperature
The graphs below show the relative activity of arylacylamidase at various temperatures and pH under the assay conditions with the p-nitroacetanilide substrate:

Calculation
Calculate arylacylamidase activity as follows:

\[ \text{U/mg} = \frac{\Delta A_{405}}{10 \times sv} \times cv \times \text{dilution} \]

where,
- \( \Delta A_{405} \) = reaction volume in mL
- \( sv \) = enzyme sample volume in mL

Assay Method

Reagents
1. Tris-HCl buffer: 50 mM, pH 7.0.
2. Stock Substrate Solution: Dissolve 3.03 g of Tris Base in 450 mL of DI water. Heat to 65 ± 5°C. With stirring, add 901 mg of p-nitroacetanilide and stir vigorously until it is dissolved. Do not over-heat. Cool the solution to 24 ± 2°C but not less than 20°C. Adjust solution to pH 8.50 ± 0.02 with 5M HCl. Absorbance at 405 nm must be ≤0.2 vs. DI water. Store in amber bottle at room temperature.
3. Diluted β-Mercaptoethanol: Add 50 µL of β-mercaptoethanol to 450 µL of DI water. Prepare fresh daily and store capped at room temperature.
4. Enzyme Diluent: Add 140 µL of Diluted β-mercaptoethanol to 100 mL of 50 mM Tris-HCl buffer, pH 7.0. Make fresh daily and store cold in a sealed container.
5. Working Reagent: Add 140 µL of Diluted β-mercaptoethanol to 100 mL of Stock Substrate Solution. Prepare fresh daily and store capped at room temperature in an amber bottle.
6. Enzyme Solution: Prepare a 10 mg/mL enzyme solution in enzyme diluent. Dilute the enzyme in same to yield an activity of approximately 0.10 to 0.15 U/mL.

Procedure
Combine 0.48 mL of Working Reagent equilibrated to 30°C with 20 µL of diluted enzyme solution in a cuvette.
Mix and measure the rate of increase in absorbance between one and three minutes at 405 nm in a spectrophotometer controlled at 30°C.
The change in absorbance should be between 0.03 and 0.07 per minute.

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