Enzymatic Assay of ARYLAMIDASE
(EC 3.4.11.2)

PRINCIPLE:

\[ \text{L-Alanine } \beta\text{-Naphthylamide} \xrightarrow{\text{Arylamidase}} \beta\text{-Naphthylamine} + \text{L-Alanine} \]

\[ \beta\text{-Naphthylamine} + p\text{-Dimethylaminocinnamaldehyde} \rightarrow \text{Schiff's Base} \] (Red Colored)

CONDITIONS: \( T = 37 ^\circ\text{C}, \) \( \text{pH} = 7.0, \) \( A_{540nm} \), Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Sodium Phosphate Buffer, pH 7.0 at 37°C
   (Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Adjust to pH 7.0 at 37°C with 1 M NaOH.)

B. 0.50 mM L-Alanine-β-Naphthylamide Solution (L-Ala-Naphth)
   (Prepare 10 ml in Reagent A using L-Alanine β-Naphthylamide, Free Base.)

C. 95% (v/v) Ethanol (EtOH)
   (Prepare 25 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

D. 260 mM Hydrochloric Acid/Ethanol Solution (HCl)
   (Prepare 25 ml in Reagent C using Hydrochloric Acid.)

E. 0.06% (w/v) p-Dimethylaminocinnamaldehyde Solution (DMAC)
   (Prepare 20 ml in Reagent C using p-Dimethylaminocinnamaldehyde.)

F. Arylamidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.05 – 0.10 unit/ml of Arylamidase in cold Reagent A.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent B (L-Ala-Naphth)</td>
<td>1.00</td>
<td>1.00</td>
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</table>

Equilibrate to 37°C. Then add:

<p>| | | |</p>
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<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
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</table>

Mix by inversion and incubate at 37°C for exactly 5 minutes. Then add:

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<tbody>
<tr>
<td>Reagent D (HCl)</td>
<td>1.00</td>
<td>1.00</td>
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</table>

Cool in an ice bath. Then add:

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</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (DMAC)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by inversion and let the color develop for approximately 10 minutes in an ice bath. Record the $A_{540}$nm for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A_{540\text{nm Test}} - A_{540\text{nm Blank}})(3.1)(df)}{(42.3)(5)(0.1)}
\]

- 3.1 = Total volume (in milliliters) of assay
- df = Dilution factor
- 42.3 = Millimolar extinction coefficient of the azo product (Schiff's Base) at 540 nm
- 5 = Time (in minutes) of assay
- 0.1 = Volume (in milliliter) of enzyme used in assay

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of L-alanine-β-naphthylamide to L-alanine and β-naphthylamine per
minute at pH 7.0 at 37°C.
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FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 100 mM sodium phosphate, 0.45 mM L-alanine-β-naphthylamide, and 0.005 - 0.01 unit arylamidase.

REFERENCE:


NOTES:

1. This value was determined by Sigma.  
2. This assay is based on the cited reference.