

**Enzymatic Assay of ARYLAMIDASE
(EC 3.4.11.2)**

PRINCIPLE:

L-Alanine β -Naphthylamide $\xrightarrow{\text{Arylamidase}}$ β -Naphthylamine + L-Alanine
 β -Naphthylamine + p-Dimethylaminocinnamaldehyde \longrightarrow Schiff's Base
(Red Colored)

CONDITIONS: T = 37°C, 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, Sigma Prod. No. S-0751. 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, Sigma Prod. No. A-2628.)
- 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, Sigma Prod. No. H-7020.)
- E. 0.06% (w/v) p-Dimethylaminocinnamaldehyde Solution (DMAC)
(Prepare 20 ml in Reagent C using p-Dimethylaminocinnamaldehyde, Sigma Prod. No. D-4506.)
- 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:

	<u>Test</u>	<u>Blank</u>
Reagent B (L-Ala-Naphth)	1.00	1.00
Equilibrate to 37°C. Then add:		
Reagent F (Enzyme Solution)	0.10	-----
Mix by inversion and incubate at 37°C for exactly 5 minutes. Then add:		
Reagent D (HCl)	1.00	1.00
Cool in an ice bath. Then add:		
Reagent F (Enzyme Solution)	-----	0.10
Reagent E (DMAC)	1.00	1.00

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

CALCULATIONS:

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

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 nm¹

5 = Time (in minutes) of assay

0.1 = Volume (in milliliter) of enzyme used in assay

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

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:

Hiwada, K., Ito, T., Yokoyama, M., and Kokubu, T. (1980)
European Journal of Biochemistry **104**, 155-165

NOTES:

1. This value was determined by Sigma.
2. This assay is based on the cited reference.
3. 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.