A-010

Bulk Enzyme Production

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

p-Nitroacetanilide + H_2O → p-Nitroaniline + Acetic acid

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

Acetaminophen + H_2O → p-Aminophenol + 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.

Used for the enzymatic determination of acetaminophen in serum

Measures the increase of p-aminophenol via secondary colorimetric chemistries

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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.

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 90.1 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.

Properties

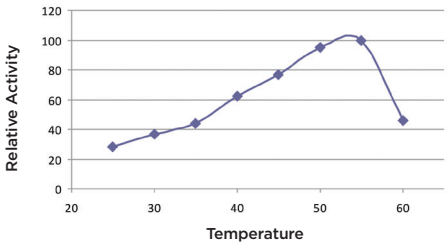
Solubility
Arylacylamidase is soluble in water and buffers.

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.

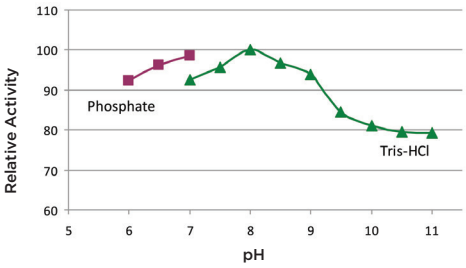
Concentration	Tris	Phosphate
20 mM, pH 8.5	98%	99%
50 mM, pH 8.5	100%	97%
100 mM, pH 8.5	94%	94%

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:

A-010 Temperature Effect



A-010 pH Effect



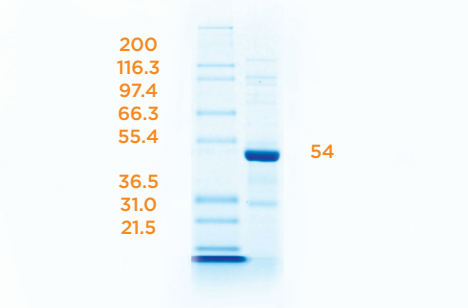
Michaelis-Menten Constant
Arylacylamidase has an apparent K_M of ≥200 mM for acetaminophen and 38 µM for p-nitroacetanilide.

pI
Arylacylamidase has an apparent pI of 4.9-5.2.

Molecular Weight
The molecular weight of arylacylamidase was determined to be ≈ 52 kDa via size exclusion chromatography and subsequent enzyme analysis.

The image below demonstrates the electrophoretic separation of a sample from a lot of arylacylamidase. Protein standard markers are shown on the left.

The major protein migrates as a single polypeptide chain of 54 kDa. Mass spectrophotometric analysis confirms the presence of a 51 kDa protein.



Calculation
Calculate arylacylamidase activity as follows:

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

where,
cv = reaction volume in mL
sv = enzyme sample volume in mL

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