# Enzyme Assay of ARYLAMINE ACETYLTRANSFERASE
# (EC 2.3.1.5)

**PRINCIPLE:**

Acetyl-CoA + Dye $\xrightarrow{AAT}$ CoASH + Acetylated Dye

Abbreviations used:
Acetyl-CoA = Acetyl Coenzyme A
Dye = p-Nitroaniline
AAT = Arylamine Acetyltransferase
CoASH = Coenzyme A

**CONDITIONS:**

T = 25°C, pH = 8.0, $A_{400nm}$, Light path = 1 cm

**METHODS:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Potassium Phosphate Buffer, pH 8.0 at 25°C (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.0 at 25°C with 1 M KOH.)

B. 100 mM ß-Mercaptoethanol Solution (ß-ME) (Prepare 5 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

C. 30 mM Ethylenediaminetetraacetic Acid Solution (EDTA) (Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 1.5 mM p-Nitroaniline Solution (Dye) (Prepare 5 ml in deionized water using p-Nitroaniline, Sigma Prod. No. N-2128.)

E. 6.0 mM Acetyl Coenzyme A Solution (Acetyl CoA)(Prepare 1 ml in deionized water using Acetyl Coenzyme A (C2:0), Sodium Salt, Sigma Prod. No. A-2056. **PREPARE FRESH.**)

**REAGENTS:** (continued)

F. Arylamine Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 100 mg/ml of Arylamine Acetyltransferase in cold Reagent A.)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 1.50 | 1.50 |
| Reagent B (ß-ME) | 0.15 | 0.15 |
| Reagent C (EDTA) | 0.10 | 0.10 |
| Reagent D (Dye) | 0.20 | 0.20 |
| Reagent F (Enzyme Solution) | 1.00 | 1.00 |

Mix by inversion and equilibrate to 25°C. Monitor the $A_{400nm}$ until constant (approx. 4 minutes) using a suitably thermostatted spectrophotometer. Then add:

| | Test | Blank |
|---|---|---|
| Reagent E (Acetyl CoA) | 0.05 | ------ |
| Deionized Water | ------ | 0.05 |

Immediately mix by inversion and record the increase in $A_{400nm}$ for approximately 5 minutes. Obtain the r $A_{400nm}$/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{400nm} \text{ Test} - \text{r } A_{400nm} \text{ Blank})(1000)(3)(df)}{(11.19)(1)}$$

1000 = Conversion from µmoles to nanomoles
3 = Volume (in milliliters) of assay
df = Dilution factor
11.19 = Millimolar extinction coefficient of acetylated dye

1 = Volume (in milliliters) of enzyme used

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

**CALCULATIONS:** (continued)

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

**UNIT DEFINITION:**

One unit will acetylate 1.0 nanomole of p-nitroaniline per minute at pH 8 at 25°C.

**FINAL CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 83 mM potassium phosphate, 5 mM ß-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 0.1 mM p-nitroaniline, 0.1 mM acetyl coenzyme A and 100 mg of arylamine acetyltransferase.

**REAGENTS:**

Brooks, S.P.J. and Storey, K.B., (1993) *Analytical Biochemistry* **212**, 452-456

**NOTES:**

1. This assay is based on the cited reference.
2. 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.**