# Enzymatic Assay of SALICYLATE HYDROXYLASE (EC 1.14.13.1)

**PRINCIPLE:**

$$\text{Salicylate} + \text{ß-NADH} + O_2 \xrightarrow{\text{Salicylate Hydroxylase}} \text{Catechol} + \text{ß-NAD} + H_2O + CO_2$$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 30°C, pH = 7.6, $A_{340nm}$, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 20 mM Potassium Phosphate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid, pH 7.6 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.6 at 30°C with 1 M HCl.)

B. 3 mM Sodium Salicylate Solution (Salicylate)
(Prepare 10 ml in Reagent A using Salicylic Acid, Sodium Salt, Sigma Prod. No. S-3007.)

C. 5.4 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt Stock No. 340-105, in the appropriate volume of cold Reagent A. **PREPARE FRESH**.)

D. Salicylate Hydroxylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.5 unit/ml of Salicylate Hydroxylase in cold deionized water.)

## Enzymatic Assay of SALICYLATE HYDROXYLASE (EC 1.14.13.1)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 2.70 | 2.70 |
| Reagent B (Salicylate) | 0.10 | 0.10 |
| Reagent C (ß-NADH) | 0.10 | 0.10 |

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|---|---|---|
| Deionized Water | ------ | 0.10 |
| Reagent D (Enzyme Solution) | 0.10 | ------ |

Immediately mix by inversion and record the decrease in $A_{340nm}$ for approximately 5 minutes. Obtain the $\Delta A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3.0)(df)}{(6.22)(0.10)}$$

3.0 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.10 = Volume (in milliliters) of enzyme used

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

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

**UNIT DEFINITION:**

One unit will convert 1.0 µmole of salicylate and ß-NADH to catechol and ß-NAD per minute at pH 7.6 at 30°C.

# Enzymatic Assay of SALICYLATE HYDROXYLASE (EC 1.14.13.1)

## FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 19 mM potassium phosphate, 0.97 mM ethylenediaminetetraacetic acid, 0.1 mM sodium salicylate, 0.18 mM ß-NADH, and 0.025 - 0.05 unit salicylate hydroxylase.

## REFERENCE:

White-Stevens, R.H. and Kamin, H. (1972) *Journal of Biological Chemistry* **247**, 2358-2370.

## NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.