S-010

Bulk Enzyme Production

Salicylate Hydroxylase

E.C. 1.14.13.1

Used for the enzymatic determination of salicylate in serum

Measures the decrease in NADH at 340 nm wavelength

Salicylate hydroxylase catalyzes the hydroxylation and simultaneous decarboxylation of salicylate to catechol:

$$\text{Salicylate} + \text{NADH} + O_2 + 2H^+ + \rightarrow \text{Catechol} + \text{NAD} + H_2O + CO_2$$

Salicylate hydroxylase has also been used in the determination of NADH as described in the U.S. Patent 4,394,444. In this case benzoate is used as a pseudo-substrate.

$$\text{Benzoate} + \text{NADH} + H^+ + O_2 \rightarrow \text{Benzoate} + NAD^+ + H_2O_2$$

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Specifications

Form
Yellow lyophilized powder.

Activity
≥ 3.5 U/mg powder.

Contaminants
NADH oxidase	≤ 0.7% SH activity

Unit
The amount of enzyme which catalyzes the conversion of 1 µmole of NADH per minute at 37°C under the conditions given in the assay method.

Assay Method

Reagents
1. Potassium Phosphate buffer: 0.02 M, pH 7.6.
2. Substrate Solution: Dissolve 21.3 mg sodium salicylate, 112 mg NADH, and 372 mg $Na_2EDTA \cdot 2H_2O$ in 900 mL of 0.02 M phosphate buffer, pH 7.6. Adjust the pH to 7.6 if necessary. Add sufficient 0.02 M phosphate buffer, pH 7.6 to bring the volume to 1000 mL. The A_{340} should measure 0.90 ± 0.05. The solution should be stored at 5°C.
3. Enzyme diluent: Prepare a solution of 0.05% sodium azide in DI H_2O.
4. Enzyme Solution: Prepare a 1 mg/mL enzyme solution in enzyme diluent. Dilute the enzyme in same to yield an activity of approximately 0.3 U/mL. Keep the diluted enzyme chilled.

Procedure
Combine 3.0 mL Substrate Solution at 37°C with 0.10 mL of diluted enzyme in a cuvette.

Mix and measure the rate of decrease in absorbance at 340 nm wavelength in a spectrophotometer controlled at 37°C.

The change in absorbance should be between 0.03 and 0.09 per minute.

Test and subtract a reagent blank by substituting enzyme diluent for diluted enzyme.

Properties

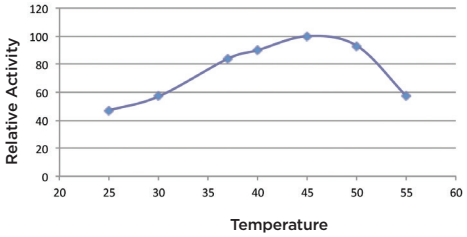
Solubility
Salicylate hydroxylase is soluble in water and buffers.

Thermal Stability
A 2 mg/mL solution in 0.05% sodium azide retains 42% of activity after 28 days of storage at 37°C and retains 99% activity after 28 days of storage at 5°C.

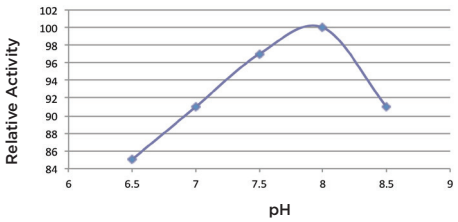
pH Stability
Salicylate hydroxylase retains >95% activity for 24 hours at 4°C in 0.1 M potassium phosphate buffer at pH 7.5, 8.0 and 8.5.

Optimum pH and Temperature
The graphs below show the relative activity of salicylate hydroxylase at various temperatures and pH under the assay conditions:

S-010 Temperature Effect



S-010 pH Effect



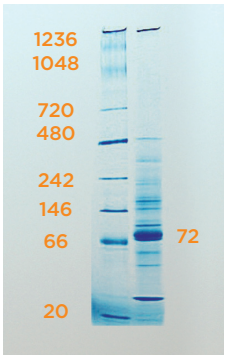
Michaelis-Menten Constant
Salicylate hydroxylase has an apparent K_M of: 2.7 x 10-6 M for salicylate
17 x 10-6 M for NADH
2.0 x 10-3 M for benzoate

pI
Salicylate hydroxylase has an apparent pI of 4.9.

Effect of Buffers
Chloride and other inorganic monovalent anions inhibit salicylate hydroxylase and are not recommended. The use of sulfate or acetate with Tris buffers may provide better stability and performance.

Molecular Weight
The molecular weight of salicylate hydroxylase was determined to be ≈ 72 kDa via size exclusion chromatography and subsequent enzyme analysis.

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



The major protein migrates as a 72 kDa protein. It consists of two subunits following reduction. Mass spectrophotometric analysis confirms the presence of a 73 kDa protein.

Calculation
Calculate salicylate hydroxylase activity as follows:

$$U/mg = \frac{(\Delta A_{340} \text{ test} - \Delta A_{340} \text{ blank}) \times cv \times dilution}{6.22 \times sv}$$

where,

cv = reaction volume in mL

sv = enzyme sample volume in mL

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