



# SALICYLATE Enzyme Assay Kit



Instructions for Use



For *in vitro* diagnostic use only



Store in DARK at 2 to 8°C

DO NOT FREEZE



**K9001**

|   |           |
|---|-----------|
| Enzyme Reagent  | 3 x 10mL  |
| NADH Reagent  | 1 x 15mL  |
| Aqueous Calibrator (Standard)<br>(1.50 mmol/L / 207 mg/L) | 1 x 3.0mL |



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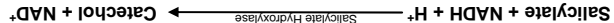
## 1. Clinical Application

Salicylate (aspirin) is a common non-steroidal drug used for its analgesic and anti-inflammatory properties. Ingestion of large amounts of salicylate leads to disturbances of the central nervous system and to gastrointestinal problems, encephalopathy and renal failure<sup>1</sup>. Due to its accessibility, accidental or intentional ingestion by children and adults represents a major poisoning problem<sup>2,3,4</sup>. Salicylate intoxication represents an acute medical emergency and rapid diagnosis and quantitation of the drug is necessary to assess effective patient management. Serum concentrations in excess of 4.4 mmol/L (607 mg/L) are usually lethal.

Salicylate has traditionally been measured by the 'Trinder' reaction<sup>5</sup>, which is based on the interaction between salicylate and ferric ions. This test is not specific. This enzymatic Salicylate Assay provides a rapid, specific and simple method for salicylate determination.

## 2. Principle of the Assay

Salicylate Hydroxylase catalyses the conversion of salicylate and NADH to catechol and NAD in the presence of oxygen. The resulting decrease in absorbance at 340 nm, due to the conversion of NADH to NAD, is directly proportional to the concentration of salicylate in the sample:



## 3. Sample

Fresh, clear, unhaemolysed human serum is the recommended specimen. Plasma collected in EDTA, heparin, fluoride oxalate or citrate collection tubes may be used.

## 4. Use of the Kit

Reagents are supplied ready to use. The reagents should be clear; significant turbidity would indicate some deterioration in the reagent. The reagents are stable until the expiry date stated when stored at 2 - 8°C.

## Conditions

1. Measure absorbance at 340nm ( $t = 10$ ).
2. Incubate for 10 minutes at 18 - 25°C.
3. Add 300µL of NADH Reagent into each cuvette. The addition of enzyme reagent. (t = 0). It is important to measure the absorbance immediately after mixing in turn, mixing and measure the absorbance at 340nm cuvette in turn, add 600µL of Enzyme Reagent into each cuvette to start the reaction, add 600µL of NADH Reagent into each cuvette.
4. Add 300µL of NADH Reagent into each cuvette.
5. To start the reaction, add 600µL of Enzyme Reagent into each cuvette in turn, mixing and measure the absorbance at 340nm (t = 0). It is important to measure the absorbance immediately after the addition of enzyme reagent.

## Assay Procedure

1. Label cuvettes for each sample, calibrator, control or blank and add 20µL of each sample to the respective cuvettes or 20µL of water for the blank.
  2. Add 300µL of NADH Reagent into each cuvette.
  3. To start the reaction, add 600µL of Enzyme Reagent into each cuvette in turn, mixing and measure the absorbance at 340nm (t = 0). It is important to measure the absorbance immediately after the addition of enzyme reagent.
  4. Incubate for 10 minutes at 18 - 25°C.
  5. Measure absorbance at 340nm ( $t = 10$ ).
- It is recommended that samples be tested in duplicate. Experience with the technique may, however, suggest that single determinations of samples are adequate at the discretion of the user. A salicylate calibrator (standard) is included with the reagents and should be used to calibrate the manual procedure with each run.
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4. Incubate for 10 minutes at 18 - 25°C.
5. Measure absorbance at 340nm ( $t = 10$ ).

## Equipment and Reagents not Supplied

A Spectrophotometer capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340nm. The bandwidth should be 10nm or less, stray light 0.5% or less and the wavelength accuracy within 2nm.

1cm cuvette or a flow cell capable of transmitting light at 340nm.

Positive displacement pipettes.

Distilled or deionised water.

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2. Add 300µL of NADH Reagent into each cuvette.

3. To start the reaction, add 600µL of Enzyme Reagent into each cuvette in turn, mixing and measure the absorbance at 340nm (t = 0). It is important to measure the absorbance immediately after the addition of enzyme reagent.

4. Incubate for 10 minutes at 18 - 25°C.

5. Measure absorbance at 340nm ( $t = 10$ ).

## Automated Analyser

|                   |                      |                   |
|-------------------|----------------------|-------------------|
| Manual            | 340nm                | Wavelength        |
| 18 - 25°C or 37°C | 10mm                 | Pathlength        |
| Endpoint          | Endpoint             | Mode              |
| 300 sec           | 10 min for 18 - 25°C | Reaction time     |
| 37°C              | 5 min for 37°C       | Sample Volume     |
| 340nm             | 20µL                 | NADH Rgt Volume   |
| 340nm             | 300µL                | Enzyme Rgt Volume |
| 340nm             | 600µL                | Total Volume      |

\* 410nm or similar if using a secondary wavelength.



## Calculation of Results

Sample conc. (mmol/L) =

$$\frac{\text{Sample (T0 - T10) } 340\text{nm} - \text{Blank (T0 - T10) } 340\text{nm} \times \text{CAL}}{\text{Sample (T0 - T10) } 340\text{nm} - \text{Blank (T0 - T10) } 340\text{nm}}$$

Where: Sample = sample absorbance

Blank = Blank absorbance (water replacing sample)

Std = Standard absorbance

CAL = Calibrator concentration (1.5mmol/L or 207mg/L)

Example

$$\frac{(2.494 - 2.083) - (2.364 - 2.328) \times 1.50 \text{ mmol/L}}{(2.333 - 2.095) - (2.364 - 2.328)}$$

$$= \frac{0.411 - 0.036 \times 1.50 \text{ mmol/L}}{0.238 - 0.036}$$

= Salicylate concentration = 2.78 mmol/L (383.6mg/L)

Conversion: mmol/L x 138 = mg/L; mg/L / 138 = mmol/L

These instructions describe how to perform the assay manually

with a spectrophotometer. However, it is also possible to run this assay on a range of automated analysers. Protocols are available for most clinical chemistry automates. The general parameters

and the conditions have been validated by Cambridge Life

Sciences. For further information, please contact Cambridge Life

Sciences or your local distributor. Current versions of the protocols can be obtained from our website;

[www.clinicaldiagnostics.com](http://www.clinicaldiagnostics.com) and it is advised that the user confirms that the current version is being used.

## Quality Control

Good Laboratory Practice requires that quality control specimens be

included in every run to monitor assay performance. The quality control samples should be assayed repeatedly to establish mean values and

working ranges.

## Linearity

The method is linear up to a sample Salicylate concentration of 10.0 mmol/L (1380 mg/L).

## Recovery

Analysis of serum samples to which known amounts of Salicylate had been added gave a mean recovery of 101.7% (range 97.1 to 106.7%).

## Interferences

The following substances when added at a concentration of 500mg/L to serum containing salicylate showed no interference:

|                   |                      |                   |
|-------------------|----------------------|-------------------|
| acetaminophen     | acetylsalicylic acid | sodium EDTA       |
| ibuprofen         | α-ketobutyric acid   | methyl salicylate |
| phenol            | salicylamide         | sodium benzoate   |
| sodium oxalate    | theophylline         | uric acid         |
| n-acetyl cysteine | amitriptyline        | caffeine          |
| promethazine      | phenytoin            | diphenhydramine   |
| imipramine        | indomethacin         | tolbutamide       |
| phenethidine      |                      |                   |

p-aminosalicylic acid and 2, 5 dihydroxybenzoic acid (gentisic acid) are measured by this assay at a concentration of 500mg/L.

No interference was observed from bilirubin at 0.5mg/mL, haemoglobin at 5mg/mL and ascorbate at 2mg/mL. Lipids up to 500mg/dL maybe measured, above this it is recommended to use Lipoclear tubes taking into account the 20% dilution effect. No interference was found using heparin, EDTA, fluoride oxalate and citrate blood collection tubes.

## Safety Precautions

For *in vitro* diagnostic use only.

For Professional use only.

No special precautions are needed with these reagents. However, general care in reagent handling is recommended.

Used samples, controls and pipette tips should be handled as clinical

waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is

recommended that gloves be worn when handling such items.

Safety data sheets are available upon request.

## 5. Performance

### Precision

Typical precision for the assay is as follows:

| C.V. | S.D.  | Conc  | (mmol/L) | (mg/L) | (mmol/L) | (mg/L) | (%) |
|------|-------|-------|----------|--------|----------|--------|-----|
| 6.3  | 2.35  | 37.3  | 0.017    | 0.091  | 163.9    | 12.62  | 7.7 |
| 5.6  | 27.68 | 497.0 | 0.201    | 0.201  | 497.0    | 27.68  | 5.6 |
| 1.1  | 3.46  | 309.4 | 0.025    | 0.025  | 309.4    | 3.46   | 1.1 |
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