Technical Bulletin

Salicylate Assay Kit

Catalogue number MAK534

Product Description

Salicylate is a salt or ester of salicylic acid and can be found naturally in some plants. It is also a metabolic byproduct of aspirin (acetylsalicylic acid) and salicylate concentrations are often tested in blood or urine in cases of suspected overdose. Salicylic acid is commonly used in skincare products as an exfoliating ingredient, and in other consumer products as a preservative.

The Salicylate Assay Kit provides a convenient and reliable means to measure salicylate. In the assay, salicylate complexes with ferric chloride to create a colored compound that can be measured at 560 nm. This assay can be used with a variety of samples and is simple, sensitive, and adaptable to high-throughput screening.

The linear detection range of the kit is 0.8 mM (10.9 mg/dL) to 20 mM (274.2 mg/dL) salicylate The kit is suitable for salicylate activity determination in biological samples such as serum, plasma, urine, as well as for consumer products e.g., beauty products and mouthwash.

Salicylate was spiked into rat plasma, rat serum, human serum, and human plasma, and human urine, and was assayed using the 96-well plate assay protocol and was determined to have acceptable % recovery. EDTA, Heparin, Citrate, and RIPA buffer do not interfere with this assay. Beauty products and mouthwash are also compatible with this kit.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent 20 mL
  Catalogue Number MAK534A
- Standard (100 mM Salicylate) 200 µL
  Catalogue Number MAK534B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader and Cuvettes for procedure using cuvette.
- Clear flat-bottom 96-well plates and 96-well plate absorbance (590nm) reader for procedure using 96-well plate. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Reagent: Shake well before use.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Serum, plasma, urine and other liquid Samples can be used directly.

Samples should be transparent and precipitate free. If Samples are cloudy or have precipitates, centrifuge 5 minutes at 14,000 x g and use clear supernatant for assay.

If Samples contain high levels of proteins (that is. plasma), they may precipitate out of solution due to acidity of Reagent. In this case, combine the Sample and the Reagent in a microcentrifuge tube and mix well, then centrifuge it and use the supernatant in the assay.

Standard Curve Preparation

1. Prepare 200 µL of 20 mM Premix by mixing 40 µL of the 100 mM Standard and 160 µL of purified water.
2. Further dilute standards in 1.5 mL centrifuge tubes as described in the Table 1.

Table 1
Preparation of Standards

Well	Premix+ purified water	Salicylate (mM)
1	100 µL + 0 µL	20
2	65 µL + 35 µL	13
3	30 µL + 70 µL	6
4	0 µL + 100 µL	0

Measurement

96-well plate:

1. Transfer 20 µL of standards into separate wells of a clear, flat-bottom 96-well plate.
2. Transfer 20 µL of a sample into a single well, as well as 20 µL into another well for the sample blank.
3. Add 180 µL of Reagent to each Standard and Sample well.
4. Add 180 µL of deionized water to the Sample Blank well.
5. Tap plate lightly to ensure the contents of the wells are mixed evenly. Read optical density at 560 nm (500-600)

Cuvette:

Note: With a 1 mL cuvette, the linear detection range changes to 0.4 mM - 10 mM because cuvettes have higher pathlengths.

1. Dilute standards by three-fold by adding 40 µL standard to 80 µL purified water in tubes.
2. Transfer 100 µL standards and samples to cuvettes.
3. Add 300 µL Reagent and 600 µL purified water to a final volume of 1000 µL in each cuvette.
4. Read optical density at 560 nm (500-600) of each sample or standard.

Results

1. Subtract the Blank value from the standard values.
2. Plot the ΔOD against standard concentrations and determine the slope.
3. Calculate the salicylate concentration of sample using the below equation:

Salicylate (mM) =

$$\frac{OD_{Sample}-OD_{Sample\ Blank}}{OD_{Standard}-OD_{Sample}} \times \frac{[Standard]}{4} \times n$$

Where:

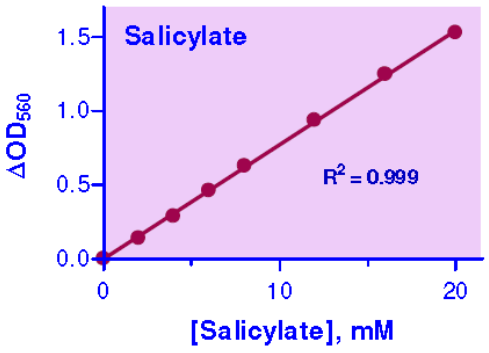
OD_{SAMPLE} and $OD_{SAMPLE\ BLANK}$ are optical density readings at 560nm of the Sample and Sample Blank respectively.

n is the dilution factor

concentration of a sample is higher than 20 mM, dilute sample in water and repeat the assay. Multiply the result by the dilution factor n.

Conversions: 1 mM Salicylate is 137.11 ppm, 13.7 mg/dL, 0.014% w/v.

Figure 1.
Typical Salicylate Standard Curve in purified water



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