Salicylate Assay Kit (BA0225)

Product Datasheet | SKU BA0225

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. In this assay, salicylate complexes with ferric chloride to create a coloured compound measured at 560 nm; the assay can be used with a variety of samples and is simple, sensitive and adaptable to high-throughput screening.

Specifications

Detection Method	Colorimetric; read at 560 nm (500-600 nm)
Detection Range	0.8 mM (10.9 mg/dL) to 20 mM (274.2 mg/dL) salicylate (96-well)
Sample Type	Serum, plasma, urine, beauty products, mouthwash
Species Reactivity	All
Assay Time	Approximately 20 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store kit at 2 degrees C to room temperature.
Shelf Life	12 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent	20 mL	2 degrees C to RT
Standard (100 mM salicylate)	200 uL	2 degrees C to RT

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0225	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. For sample preparation, ensure samples are transparent and precipitate-free; if cloudy or containing precipitates, centrifuge 5 minutes at 14,000 x g and use the clear supernatant. Serum, plasma, urine and other liquid samples can be used directly, but high-protein samples may precipitate due to the acidity of the Reagent, in which case combine the sample and Reagent in a microcentrifuge tube, mix well, centrifuge and use the supernatant.
2. Prepare 200 microlitres of 20 mM Premix by mixing 40 microlitres of the 100 mM Standard with 160 microlitres of distilled water, then dilute standards in 1.5 mL centrifuge tubes as described in the standard preparation table.
3. Transfer 20 microlitres of standards into separate wells of a clear flat-bottom 96-well plate, transfer 20 microlitres of a sample into a single well and 20 microlitres into another well for the sample blank.
4. Add 180 microlitres of Reagent to each Standard and Sample well, add 180 microlitres of deionised water to the Sample Blank well and tap the plate lightly to ensure the contents are mixed evenly.
5. Read optical density at 560 nm (500-600 nm).

6. Cuvette procedure: dilute standards three-fold by adding 40 microlitres of standard to 80 microlitres of water in tubes, transfer 100 microlitres of standards and samples to cuvettes, add 300 microlitres of Reagent and 600 microlitres of water to a final volume of 1000 microlitres in each cuvette, then read optical density at 560 nm (500-600 nm). Note: with a 1 mL cuvette the linear detection range changes to 0.4 mM - 10 mM because cuvettes have higher pathlengths.

Standard Preparation (96-well)

No	Premix + dH2O	Salicylate (mM)
1	100 uL + 0 uL	20
2	65 uL + 35 uL	13
3	30 uL + 70 uL	6
4	0 uL + 100 uL	0

Calculation

Subtract the Blank value (Standard #4) from the standard values and plot the delta-OD against standard concentrations. Determine the slope and calculate the salicylate concentration as: [Salicylate] = [(OD_SAMPLE - OD_SAMPLE BLANK) / (OD_STANDARD - OD_SAMPLE)] x ([Standard]/4) x n (mM), where OD_SAMPLE and OD_SAMPLE BLANK are the OD560 readings of the Sample and Sample Blank respectively and n is the dilution factor. Note: if the calculated salicylate concentration is higher than 20 mM, dilute the sample in water and repeat, multiplying the result by the dilution factor n. Conversions: 1 mM Salicylate is 137.11 ppm, 13.7 mg/dL, 0.014% w/v.

Notes

- Samples should be transparent and precipitate-free; briefly centrifuge the Standard tube before opening.
- Salicylate has been shown to have acceptable percentage recovery when spiked into rat and human serum, plasma and urine, and EDTA, heparin, citrate and RIPA buffer do not interfere with this assay; beauty products and mouthwash are also compatible.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.