

## QuantiChrom™ Salicylate Assay Kit (DSALY-100)

### Quantitative Colorimetric Determination of Salicylate

#### 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.

BioAssay Systems' salicylate detection 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.

#### KEY FEATURES

**Fast and sensitive.** Linear detection range: 0.8 mM (10.9 mg/dL) to 20 mM (274.2 mg/dL) salicylate with 20  $\mu$ L sample (96-well).

**Convenient.** The procedure involves adding a single working reagent.

**High-throughput.** "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well or 384-well plate assay for thousands of samples per day.

#### APPLICATIONS

**Direct Assays:** salicylate in biological samples (e.g. serum, plasma, urine)

**Consumer Products:** salicylate in beauty products, mouthwash, etc.

#### KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

**Reagent:** 20 mL      **Standard:** 200  $\mu$ L (100 mM salicylate)

**Storage conditions.** The kit is shipped at room temperature (RT). Store kit at 2°C-RT.

**Shelf life:** 12 months after receipt.

**Precautions:** Reagents are for research use only. Briefly centrifuge Standard tube before opening. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

**Sample Preparation:** (1) Samples should be transparent and precipitate-free. If samples are cloudy or have precipitates, centrifuge 5 min at 14,000 x g and use clear supernatant for assay. (2) Serum, plasma, urine, and other liquid samples can be used directly. (3) If samples contain high levels of proteins (i.e. 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.

##### Procedure using 96-well plate

- Standards.** Prepare 200  $\mu$ L of 20 mM Premix by mixing 40  $\mu$ L of the 100 mM Standard and 160  $\mu$ L of dH<sub>2</sub>O. Dilute standards in 1.5-mL centrifuge tubes as described in the Table below.

No	Premix + dH <sub>2</sub> O	Salicylate (mM)
1	100 $\mu$ L + 0 $\mu$ L	20
2	65 $\mu$ L + 35 $\mu$ L	13
3	30 $\mu$ L + 70 $\mu$ L	6
4	0 $\mu$ L + 100 $\mu$ L	0

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

##### Procedure using Cuvette

- Dilute standards three fold by adding 40  $\mu$ L standard to 80  $\mu$ L dH<sub>2</sub>O in tubes. Transfer 100  $\mu$ L standards and samples to cuvettes. Add 300  $\mu$ L Reagent and 600  $\mu$ L dH<sub>2</sub>O to a final volume of 1000  $\mu$ L in each cuvette.
- Read optical density at 560 nm (500-600) of each sample or standard.

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

#### 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 of Sample as follows:

$$[\text{Salicylate}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{SAMPLE BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{SAMPLE}}} \times \frac{[\text{Standard}]}{4} \times n \text{ (mM)}$$

$\text{OD}_{\text{SAMPLE}}$  and  $\text{OD}_{\text{SAMPLE BLANK}}$  are the OD<sub>560</sub> readings of the Sample and Sample Blank, respectively.  $n$  is the dilution factor.

**Note:** If the calculated salicylate 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.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes

##### Procedure using 96-well plates:

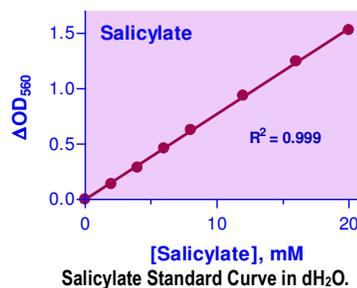
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

##### Procedure using cuvette:

Spectrophotometer and Cuvettes for measuring OD at 500-600nm.

#### SAMPLE COMPATIBILITY

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.



#### LITERATURE

- Levy, G. (1980). Clinical pharmacokinetics of salicylates: A re-assessment. *British Journal of Clinical Pharmacology*, 10(S2).
- Zhang Y, Li X. (2019). Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr Opin Plant Biol.* 50:29-36.
- Pearlman BL, Gambhir R (2009). Salicylate intoxication: a clinical review. *Postgrad Med.* 121(4):162-8.

