Technical Bulletin

Salicylate Assay Kit (Colorimetric)

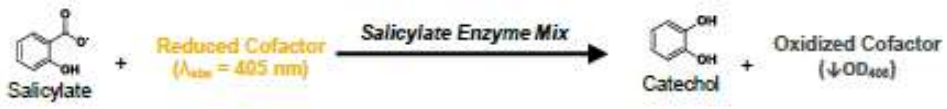
Catalog Number MAK415

Product Description

Salicylates (salts of 2-hydroxybenzoic acid) are common non-steroidal anti-inflammatory drugs (NSAIDs) with anti-pyretic and mild analgesic effects. Originally discovered in willow tree bark, salicylate has been used in an ethnopharmacological context for millennia to treat inflammation and fever. The most frequently used modern salicylate drug is aspirin (acetylsalicylic acid), which is rapidly hydrolyzed to salicylate in both the gastrointestinal tract and bloodstream. Salicylate acts as a weak inhibitor of the proinflammatory COX-2 enzyme, which is thought to underlie its anti-inflammatory effect in chronic aspirin therapy. However, supratherapeutic blood salicylate levels can cause severe intoxication and poisoning. Serum salicylate levels are monitored in people taking chronic high-dose aspirin for arthritis and in suspected cases of aspirin overdose. Therapeutic serum levels for salicylate range from 50-250 µg/mL (0.36 – 1.8 mM) in individuals on chronic aspirin therapy. Levels over 300 µg/ml (2.17 mM) are considered toxic, resulting in dose-dependent and potentially lethal symptoms such as tinnitus/deafness, lethargy/coma, seizures, and metabolic acidosis.

The Salicylate Assay Kit is a microplate-based assay suitable for high-throughput applications that allows for rapid quantification of salicylate levels in biological fluids. In the assay, salicylate is enzymatically metabolized to catechol, with concomitant oxidation of a cofactor, resulting in a decrease in absorbance at 405 nm that is proportional to the concentration of salicylate present. The assay is not affected by other NSAIDs and has a limit of detection of 0.19 mM with a reliable linear range from 1 - 20 nmole salicylate per well (corresponding to 0.2 - 4 mM salicylate in undiluted samples).

The kit is suitable for the estimation of salicylate concentration in plasma or serum.



Components

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

- Salicylate Assay Buffer 25 mL
  Catalog Number MAK415A
- Cofactor Solution 1 vial
  Catalog Number MAK415B
- Salicylate Enzyme Mix 1 vial
  Catalog Number MAK415C
- Matrix Replicator 500 µL
  Catalog Number MAK415D
- Salicylate Standard 1 vial
  Catalog Number MAK415E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Microcentrifuge capable of RCF ≥10,000 × *g*

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Salicylate Assay Buffer: Ready to use. Warm to room temperature prior to use.

Cofactor Solution: Reconstitute vial with 550 µL of purified water. Divide into aliquots and store at -20 °C, **protected from light.** Avoid repeated freeze/thaw cycles.

Salicylate Enzyme Mix: Reconstitute vial with 220 µL of Salicylate Assay Buffer. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

Matrix Replicator: Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Prior to use, warm solution to room temperature.

Salicylate Standard: Reconstitute vial with 220 µL of purified water for a 20 mM stock solution. Store at -20 °C, stable for 4 freeze/thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Collect serum or plasma samples by standard methods with the following considerations:
   a. Serum: use off-the-clot serum (collected in tubes that are free of anticoagulants).
   b. Plasma should be collected using K_2EDTA or lithium/sodium heparin.
   c. Lipemic or turbid samples should be clarified by filtration through a 0.2 µm syringe filter or by centrifugation at 10,000 x *g* for 5 minutes at room temperature in order to separate lipid globules.
   d. Do not use serum or plasma that is hemolyzed or contaminated with red blood cells. Hemoglobin has a sharp absorbance peak in the 400 – 425 nm region and will interfere with the assay.
2. Add 5 µL of undiluted serum or plasma sample to desired Sample well(s) in a clear, flat bottom 96-well plate.
3. For unknown samples, perform a pilot experiment to ensure readings are within the standard curve range. Samples that are outside of the standard curve range should be diluted at a 1:1 ratio with Matrix Replicator and retested (use 5 µL of the diluted sample per well).
4. Adjust the total volume of all sample wells to 100 µL/well with Salicylate Assay Buffer.

Standard Curve Preparation

1. Prepare a 2 mM salicylate solution by adding 20 µL of the 20 mM Salicylate Standard to 180 µL of Salicylate Assay Buffer. Prepare salicylate standards according to Table 1, mix well.

Table 1.
Preparation of Salicylate Standards

Well	2 mM Salicylate Standard	Salicylate Assay Buffer	Salicylate (nmol/ well)
1	0 µL	95 µL	0
2	2 µL	93 µL	4
3	4 µL	91 µL	8
4	6 µL	89 µL	12
5	8 µL	87 µL	16
6	10 µL	85 µL	20

2. Add 5 µL of the Matrix Replicator to each Standard well, **including** the 0 nmol/well Standard (Reagent Blank).

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed. For each well (Standard and Sample), prepare 100 µL of Enzymatic Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Enzymatic Reaction Mix
Salicylate Assay Buffer	93 µL
Salicylate Enzyme Mix	2 µL
Cofactor Solution	5 µL

2. It is critical that the reaction time of both the Standard curve and Sample wells are equivalent. To minimize the lag time between wells, use a multichannel pipette and reservoir, and add 100 µL of Enzymatic Reaction Mix to all Sample and Standard curve wells, bringing the final volume to 200 µL /well.
3. Incubate the plate for 10 minutes at room temperature.

Measurement

Following the 10 minute incubation time, measure the absorbance of all sample and standard curve wells at 405 nm (A_{405}) in endpoint mode.

Results

1. For the Salicylate Standard curve, subtract the Reagent Blank (0 nmol/well Standard) absorbance reading from each of the Standard readings to determine the net change in absorbance:

   $\Delta A_{405} = (A_{405\ Standard}) - (A_{405\ Blank})$

2. Construct a plot of Salicylate Standard amount (nmol/well) versus the absolute value of the net change in absorbance ($|\Delta A_{405}|$) and calculate the slope of the Salicylate Standard curve.

3. For Samples (S), calculate the corrected sample absorbance (AC) by subtracting the Reagent Blank (0 nmol/well Standard) from the Sample absorbance reading and take the absolute value:

   $AC = |(A_{405\ Sample}) - (A_{405\ Blank})|$

4. Apply the *AC* value to the Salicylate Standard curve to get B nmol of salicylate in the Sample well.

   Salicylate Concentration (nmol/µL or mM) =

   $(B/V) \times D$

where:

B = Amount of salicylate, calculated from the standard curve (in nmoles)

V = Volume of sample added to the well (5 µL)

D = Sample dilution factor (if applicable; D = 1 for undiluted samples)

Figure 1.
Typical Salicylate Standard Curve. Salicylate concentration is directly proportional to the decrease in absorbance measured at 405 nm (A_{405}), which is graphed as the absolute difference in A_{405} versus the reagent blank.

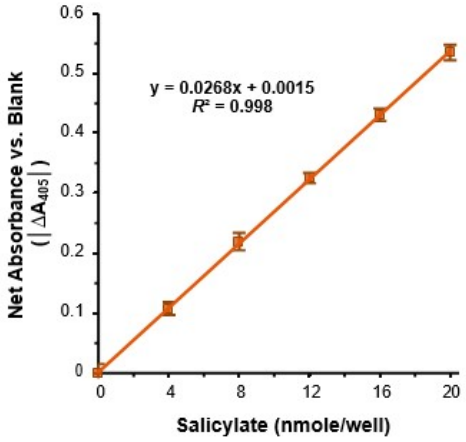
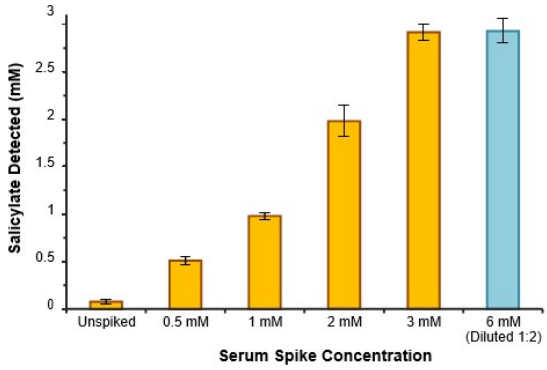


Figure 2.
Estimation of salicylate in human serum. Normal (drug-free) "off-the-clot" pooled serum was split into aliquots and spiked with either 0.5 mM, 1.0 mM, 2.0 mM or 3.0 mM salicylate (5 µL of serum was assayed in all cases). Mean salicylate concentrations detected in the spiked samples were 0.51 mM, 0.98 mM, 1.98 mM and 2.91 mM respectively (mean spike recovery rates across all spiked concentrations ranged from 97 - 102%). In the 6 mM sample, pooled human serum spiked with 6.0 mM salicylate was diluted at a 1:1 ratio with the included Matrix Replicator and assayed (5 µL of pre-diluted serum). Salicylate concentration detected in the diluted sample was 2.93 mM × 2 = 5.85 mM (spike recovery of 97.6%). Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.



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