

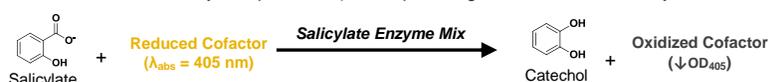
Salicylate Assay Kit (Colorimetric)

1/19

(Catalog # K494-100; 100 Assays; Store at -20°C)

I. Introduction:

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. BioVision's Salicylate Assay Kit is a high-throughput adaptable microplate-based assay that allows for rapid quantification of salicylate levels in biological fluids. In our assay, salicylate is enzymatically metabolized to catechol, with concomitant oxidation of a cofactor, resulting in a decrease in absorbance at OD = 405 nm that is proportional to the concentration of salicylate present. The assay is not affected by other NSAIDs and has an LOQ 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).



II. Applications:

- Estimation of salicylate concentration in blood

III. Sample Type:

- Human or animal plasma or serum

IV. Kit Contents:

Components	K494-100	Cap Code	Part Number
Salicylate Assay Buffer	25 ml	WM	K494-100-1
Cofactor Solution	1 vial	Blue	K494-100-2
Salicylate Enzyme Mix	1 vial	Green	K494-100-3
Matrix Replicator	500 µl	Amber	K494-100-4
Salicylate Standard	1 vial	Yellow	K494-100-5

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 405 nm)
- Clear 96-well plates with flat bottom
- Multi-channel pipette (capable of dispensing 100 µl) and reagent reservoir

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Salicylate Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **Cofactor Solution:** Reconstitute with 550 µl of ddH₂O. Divide into aliquots and store at -20°C, **protected from light**. Avoid repeated freeze/thaw cycles.
- **Salicylate Enzyme Mix:** Reconstitute 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 with 220 µl of ddH₂O for a 20 mM stock solution. Store at -20°C, stable for 4 freeze/thaw cycles.

VII. Salicylate Assay Protocol:

1. Sample Preparation:

- Collect serum or plasma samples by standard methods (see note regarding compatible blood collection tubes and anticoagulants below). 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 min at room temperature in order to separate lipid globules.
- Add 5 µl of undiluted serum/plasma sample to desired well(s) in a clear, flat bottom 96-well plate.
- Adjust the volume of all sample wells to 100 µl/well with Salicylate Assay Buffer.

Notes:

- We recommend using either “off-the-clot” serum (collected in tubes that are free of anticoagulants) or plasma collected with K₂EDTA or lithium/sodium heparin.
- Do not use serum/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.
- For unknown samples, we recommend performing 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).

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2. Standard Curve Preparation:

- Prepare a 2 mM solution of salicylate by adding 20 μl of the 20 mM Salicylate Standard stock to 180 μl of Salicylate Assay Buffer. Add 0, 2, 4, 6, 8, and 10 μl of the 2 mM working solution into a series of wells, generating 0, 4, 8, 12, 16 and 20 nmol of salicylate/well.
- Add 5 μl of the Matrix Replicator to each standard well (**including the 0 nmol/well reagent blank**).
- Adjust the volume of all Salicylate Standard wells to 100 μl /well with Salicylate Assay Buffer.

Note: To ensure accurate quantification of salicylate in samples, prepare a standard curve each time the assay is performed.

3. Reaction Mix Preparation:

- Prepare enzymatic reaction mix for sample and standard curve wells according to the table below. Make a sufficient amount of the reaction mix to add 100 μl to all assay wells.

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

- Add 100 μl of enzymatic reaction mix to all test sample and standard curve wells (**see note**), bringing the final volume to 200 μl /well.

Note: The reaction time **must be consistent for both the standard curve and sample wells**. We recommend using a multi-channel pipette and reagent reservoir for addition of Reaction Mix to minimize lag time between wells.

- Incubate the plate for 10 min at room temperature.

- Measurement:** Following 10 min incubation time, measure the absorbance of all sample and standard curve wells at 405 nm in endpoint mode.

- Calculations:** For the Salicylate Standard curve, subtract the reagent blank (0 nmol/well) absorbance reading from each of the standard readings to determine the net change in absorbance: $\Delta\text{OD}_{405} = (\text{OD}_{405})_{\text{standard}} - (\text{OD}_{405})_{\text{blank}}$. Construct a plot of Salicylate Standard amount (nmol/well) versus the absolute value of the net absorbance ($|\Delta\text{OD}_{405}|$) and calculate the slope of the standard curve. For test samples, calculate the corrected sample absorbance (A_c) by subtracting the reagent blank (0 nmol/well Salicylate Standard) from the sample absorbance reading and taking the absolute value: $A_c = |(\text{OD}_{405})_{\text{sample}} - (\text{OD}_{405})_{\text{blank}}|$. Apply the A_c value to the standard curve to get B nmol of salicylate in the sample well.

$$\text{Sample Salicylate Concentration} = \frac{B}{V} \times D = \text{nmol}/\mu\text{l} = \text{mM}$$

Where: **B** is the amount of Salicylate, calculated from the standard curve (in nmoles)

V is the volume of sample added to the well (5 μl)

D is the sample dilution factor (if applicable, $D = 1$ for undiluted samples)

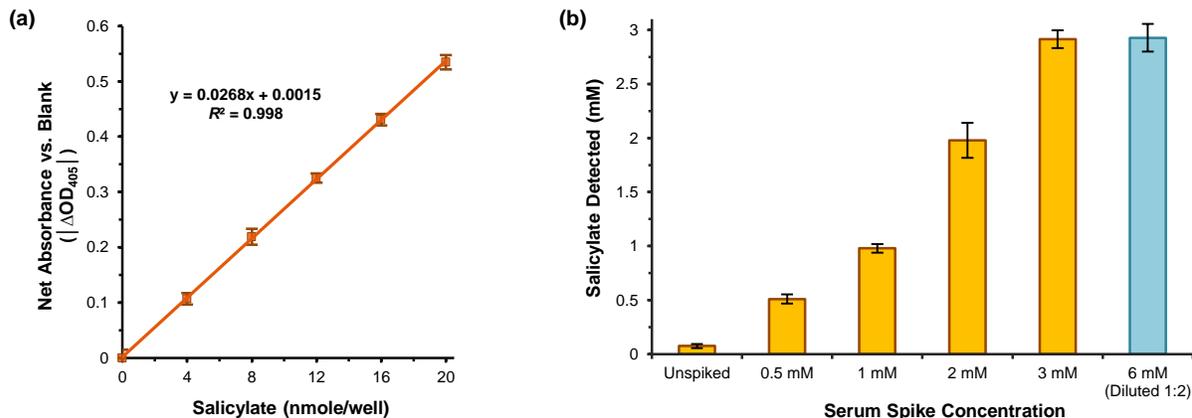


Figure: (a) Salicylate Standard curve. Salicylate concentration is directly proportional to the decrease in absorbance measured at 405 nm, which is graphed as the absolute difference in OD_{405} versus the reagent blank. (b) Estimation of salicylate in human serum. Normal (drug-free) "off-the-clot" pooled 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 \times 2 = 5.85 mM (spike recovery of 97.6%). Data are mean \pm SEM of 3 replicates, assayed according to the kit protocol.

VIII. RELATED PRODUCTS:

Cyclooxygenase (COX) Activity Assay Kit (K549)

BioSim™ Etanercept (Human Serum/Plasma) ELISA Kit (E4374)

BioSim™ Infliximab (Human Serum/Plasma) ELISA Kit (E4375)

Cyclooxygenase-1 (COX-1) Inhibitor Screening Kit (K548)

Cyclooxygenase-2 (COX-2) Inhibitor Screening Kit (K547)

Celecoxib (1574)

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