

Sialic Acid (NANA) Colorimetric/Fluorometric Assay Kit

(Catalog #K566-100; 100 Reactions; Store kit at -20°C)

I. Introduction:

Sialic acid is a generic term for the *N*- or *O*-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone. It is also the name for the most common member of this group, N-acetylneuraminic acid. Sialic acids are found widely distributed in animal tissues and to a lesser extent in other species ranging from plants and fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides. It has been shown recently that sialic acid level may be associated with developmental and pathological stages. BioVision's Sialic Acid Assay Kit provides a simple and convenient means of measuring free Sialic Acid in a variety of biological samples. The kit utilizes an enzyme coupled reaction in which free sialic acid is oxidized resulting in development of the Oxi-Red probe to give fluorescence (Ex/Em=535/587 nm) and absorbance (OD=570 nm). The kit measures sialic acid in the linear range of 0.1 to 10 nmol with a detection sensitivity ~1 μM concentration.

II. Kit Contents:

Components	K566-100	Cap Code	Part No.
Sialic Acid Assay Buffer	25 ml	WM	K566-100-1
Sialic Acid Probe (in DMSO)	0.2 ml	Red	K566-100-2A
Sialic Acid Converting Enzyme	lyophilized	Purple	K566-100-4
Sialic Acid Development Mix	lyophilized	Green	K566-100-5
Sialic Acid Standard (10 μmol)	lyophilized	Yellow	K566-100-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before the assay.

IV. Reagent Reconstitution and General Consideration:

Sialic Acid Probe: Ready to use as supplied. Warm to room temperature to melt frozen DMSO prior to use. Protect from light and moisture. Stable for 2 months at -20°C.

Sialic Acid Converting Enzyme, Development Mix: Reconstitute with 220 μl Sialic acid Assay Buffer separately. Pipette up and down to dissolve. Keep the Enzyme and Development Mix on ice during use. Aliquot and store at -20°C if they will not all be used at once. Avoid repeated freeze/thaw cycles. Use within two months.

Sialic Acid Standard: Dissolve in 100 μl dH₂O to generate 100 mM (100 nmol/μl) Sialic acid Standard Solution. Keep on ice while in use. Store at -20°C.

Ensure that the Assay Buffer is warmed to room temperature before the reaction.

V. Sialic Acid Assay Protocol:

1. Standard Curve:

For the Colorimetric Assay: Dilute 10 μl of the 100 mM Sialic Acid Standard with 990 μl dH₂O to generate 1 mM standard Sialic Acid. Add 0, 2, 4, 6, 8, 10 μl of the diluted Sialic Acid Standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 μl with Assay Buffer.

For the Fluorometric Assay: Dilute the standard to 0.1 mM (0.1 nmol/μl), then follow the same protocol as colorimetric assay. Will give 0, 0.2, 0.4, 0.6, 0.8, 1 nmol/well Standard.

2. Sample Preparation:

Samples can be tested for free sialic acid or hydrolyzed to measure bound sialic acid as well. There are a variety of hydrolysis protocols, and users should be cautious in selecting which protocol to use. Once the sample has been prepared, add samples to a 96-well plate and bring the volume to 50 μl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μl Reaction Mix containing:

	Sialic Acid Measurement	Background Control*
Assay Buffer	44 μl	46 μl
Sialic Acid Converting Enzyme	2 μl	-----
Sialic Acid Development Mix	2 μl	2 μl
Sialic Acid Probe**	2 μl	2 μl

*Pyruvate will generate background. If a significant amount of pyruvate is suspected in your sample, you may do a background control. Do pyruvate background control without Sialic Acid Converting Enzyme, which will detect only endogenous pyruvate, but not Sialic Acid. The pyruvate background should be subtracted from Sialic Acid.

** For the fluorescent assay, dilute the probe 10X to reduce background reading.

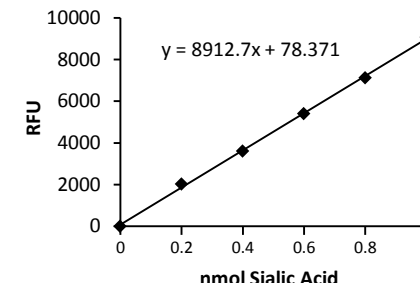
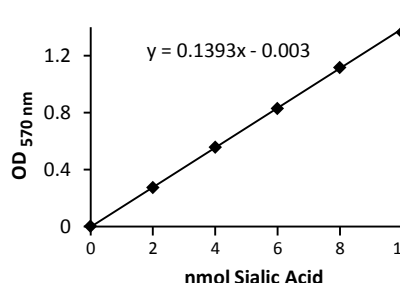
Add 50 μl of the **Reaction Mix** to each well containing the Sialic acid standard and test samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

4. Measure OD at 570 nm or fluorescence at Ex/Em 535/587 nm in a microplate reader.

5. **Calculation:** Correct background by subtracting the value derived from the 0 Sialic Acid control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot Sialic Acid standard curve. Apply sample readings to the standard curve. Sialic Acid concentrations of the test samples can then be calculated:

$$C = S_a/S_v \text{ (nmol/}\mu\text{l, or mM)}$$

Where S_a is the Sialic acid content of unknown samples (in nmol) from standard curve
 S_v is sample volume (in μl) added into the assay wells.
 Sialic acid Molecular Weight is 309.3 g/mol.



RELATED PRODUCTS:

- NAD(P)/NAD(P)H Quantification Kit
- Ascorbic Acid Quantification Kit
- Total Antioxidant Capacity (TAC) Assay Kit
- Ethanol Assay Kit
- Pyruvate Assay Kit
- Creatinine Assay Kit
- Ammonia Assay Kit
- Triglyceride Assay Kit
- Choline/Acetylcholine Quantification Kit
- Sarcosine Assay Kit
- Glycogen Assay Kit
- Phosphatase Assay Kits

- ADP/ATP Ratio Assay Kit
- Glutathione Detection Kit
- Fatty Acid Assay Kit
- Uric Acid Assay Kit
- Lactate Assay Kit I & II
- Nitric Oxide Assay Kit
- Free Glycerol Assay Kit
- Hemin Assay Kit
- Glucose Assay Kit
- L-Amino Acid Assay Kit
- Cholesterol Assay Kit
- HDL/LDL Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.