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Product Information

Sialic Acid Assay Kit

Catalog Number **MAK314**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sialic Acid is a general name for nine carbon acidic sugars with *N* or *O*-substituted derivatives. The most common member of these sugars is *N*-acetyl-neuraminic acid (NANA). Sialic acid is widely distributed throughout mammalian tissues and fluids including serum.

Sialylated oligosaccharides have been shown to exhibit antiviral properties and are also known to influence blood coagulation and cholesterol levels. The sialic acid level in body fluids is also an important marker for diagnosing cancer. Simple and direct procedures for measuring sialic acid concentrations find wide applications in research and drug discovery.

This sialic acid assay uses an improved Warren method, in which sialic acid is oxidized to formylpyruvic acid. This reacts with thiobarbituric acid to form a pink colored product. The colorimetric (549 nm)/fluorimetric (λ_{ex} = 555/λ_{em} = 585 nm) product is directly proportional to sialic acid concentration in the sample.

Components

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

Dye Reagent Catalog Number MAK314A	6 mL
10% TCA Catalog Number MAK314B	5 mL
DMSO Catalog Number MAK314C	12 mL
Oxidation Reagent Catalog Number MAK314D	10 mL
Hydrolysis Reagent Catalog Number MAK314E	10 mL
Standard, 10 mM Sialic Acid Catalog Number MAK314F	500 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Spectrophotometric or fluorescence multiwell plate reader.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Storage/Stability

The kit is shipped at room temperature. Store the components at –20 °C.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples, reagents, and standards.

Colorimetric Procedure

1. Standards – Equilibrate all components to room temperature. Prepare a 1,000 μM sialic acid Standard Premix by mixing 25 μL of the 10 mM Standard and 225 μL of ultrapure water. Dilute Standard Premix as shown in Table 1.

Table 1.
Preparation of Standards

No	Premix + water	Vol (μL)	Sialic Acid (μM)
1	100 μL + 0 μL	100	1,000
2	60 μL + 40 μL	100	600
3	30 μL + 70 μL	100	300
4	0 μL + 100 μL	100	0

   Transfer 20 μL of each standard into labeled microcentrifuge tubes and add 5 μL of 10% TCA.

2. Samples – To determine total sialic acid (TSA), samples need to be hydrolyzed to release bound sialic acid. In a microcentrifuge tube, mix 20 μL of sample, 40 μL of ultrapure water, and 40 μL of Hydrolysis Reagent. Heat at 80 °C for 60 minutes, let cool, and briefly centrifuge. Add 25 μL of 10% TCA, vortex, and centrifuge at 14,000 rpm for 10 minutes. Transfer 25 μL of supernatant into a clean tube and label it “TSA”.

   To determine free sialic acid (FSA), directly precipitate protein by mixing 40 μL of sample and 10 μL of 10% TCA. Vortex and centrifuge at 14,000 rpm for 10 minutes. Transfer 25 μL of supernatant into a clean tube and label it “FSA”.

3. Oxidation – Prepare working reagent for each tube by mixing 15 μL of Hydrolysis Reagent, 50 μL of water, and 65 μL of Oxidation Reagent. Add 125 μL of working reagent to each tube and let stand for 60 minutes at room temperature.

4. Color Reaction – Add 50 μL of Dye Reagent to each tube. Mix and heat for 10 minutes at 100 °C. Let mixture cool for 5–10 minutes. Add 100 μL of DMSO to each tube. Mix and centrifuge for 5 minutes at 14,000 rpm. Transfer 250 μL of supernatant into separate wells of a clear, flat-bottom 96-well plate.

5. Read optical density at 549 nm (540–555nm).

Fluorimetric Procedure
The fluorimetric assay is 10-fold more sensitive than the colorimetric assay. Prepare standards at 0, 30, 60 and 100 μM sialic acid in ultrapure water.

The sample treatment, oxidation, and color reaction steps are the same, except that the final reaction mixture is transferred into wells of a black, flat-bottom 96 well plate. Read fluorescence intensity (λ_{ex} = 555/λ_{em} = 585 nm).

Results
Calculations
Note: A new standard curve must be set up each time the assay is run.

Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the sialic acid concentration of Sample,

$$[\text{Sialic acid}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and water Blank (#4), respectively.
n is the sample dilution factor (n = 5 for TSA assays and n = 1 for FSA assays)

Note: if the Sample OD value is higher than that for the 1,000 μM Standard, or if the sample fluorescence intensity higher than that for the 100 μM Standard, dilute sample in water and repeat the assay. Multiply result by the fold of dilution.

Conversions: 1,000 μM NANA equals 30.9 mg/dL or 309 ppm.

References

1. Warren, L., The Thiobarbituric Acid Assay of Sialic Acids. J. Biol. Chem., **234**, 1971-1975 (1959).
2. Stefenelli, N. et al., Serum sialic acid in malignant tumors, bacterial infections, and chronic liver diseases. J. Cancer Res. Clin. Oncol., **109**(1), 55-59 (1985).
3. Sherblom, A.P. et al., Bovine serum sialic acid: age-related changes in type and content. Int. J. Biochem., **20**, 1177-1183 (1988).

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Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Fluorescence assays – black plates with clear bottoms, colorimetric assays – clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range