Sialic Acid Assay Kit

Catalog Number KA1655

100 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

- Applications:

✓ Direct Assays: sialic acid in biological samples (e.g. serum, plasma, saliva, milk).

- Features

✓ Sensitive and accurate: Use as little as 60 µL samples. Linear detection range in 96-well plate: 5 to1000 µM sialic acid for colorimetric assays and 0.5 to 100 µM for fluorimetric assays.

Background

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-acetylneuraminic 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.

Principle of the Assay

Simple and direct procedures for measuring sialic acid concentrations find wide applications in research and drug discovery. Sialic Acid Assay Kit uses an improved Warren method, in which sialic acid is oxidized to formylpyruvic acid which reacts with thiobarbituric acid to form a pink colored product. The color intensity at 549 nm or fluorescence intensity at λem/ex = 585/555 nm is directly proportional to sialic acid concentration in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Dye Reagent	6 mL
10% TCA	5 mL
DMSO	12 mL
Oxidation Reagent	10 mL
Hydrolysis Reagent	10 mL
Standard: 10 mM Sialic Acid	500 µL

Storage Instruction

Store the Standard at -20°C, all others at room temperature. Shelf life of six months after receipt.

Materials Required but Not Supplied

Pipeting devices, centrifuge tubes, centrifuge, heat block, clear flat-bottom 96-well plates, black 96-well plates (e.g. Corning Costar) and plate readers.

Precautions for Use

- Precautions

✓ Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Assay Protocol

Assay Procedure

- Colorimetric Procedure

1. Standards: Equilibrate all components to room temperature. Prepare a 1000 µM sialic acid standard Premix by mixing 25 µL of the 10 mM Standard and 225 µL distilled water dH_2O. Dilute Standard as follows.

No	Premix + dH_2O	Vol (µL)	Sialic Acid (µM)
1	100 µL + 0 µL	100	1000
2	60 µL + 40 µL	100	600
3	30 µL + 70 µL	100	300
4	0 µL + 100 µL	100	0

Transfer 20 µL standards into four labeled Eppendorf tubes, add 5 µL 10% TCA.

2. Samples treatment: To determine total sialic acid (TSA), samples need to be hydrolyzed to release bound sialic acid as follows. In an Eppendorf tube, mix 20 µL sample, 40 µL dH_2O and 40 µL Hydrolysis Reagent. Heat at 80°C for 60 min, let cool and briefly centrifuge. Add 25 µL 10% TCA, vortex and centrifuge at 14,000 rpm for 10 min. Transfer 25 µL supernatant into a clean tube and label it “TSA”.
   To determine free sialic acid (FSA), directly precipitate protein by mixing 40 µL sample and 10 µL 10% TCA. Vortex and centrifuge at 14,000 rpm for 10 min. Transfer 25 µL supernatant into a clean tube and label it “FSA”.
3. Oxidation: Prepare working reagent for each tube by mixing 15 µL Hydrolysis Reagent, 50 µL dH_2O and 65 µL Oxidation Reagent. Add 125 µL working reagent to each tube and let stand for 60 min at room temperature.
4. Color Reaction: Add 50 µL Dye Reagent to each tube. Mix and heat for 10 min at 100°C. Let cool for another 5-10 min. Add 100 µL DMSO to each tube. Mix and centrifuge for 5 min at 14,000 rpm. Transfer 250 µL supernatant into separate wells of a clear, flat-bottom 96-well plate.
5. Read optical density at 549 nm (540-555 nm).

- 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 dH_2O.

✓ 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 at λ_{ex} = 555 nm and λ_{em} = 585 nm.

Data Analysis

Calculation of Results

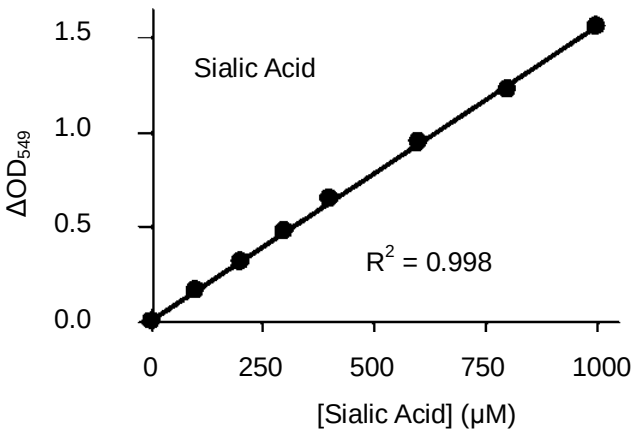
Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the sialic acid concentration of Sample,

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

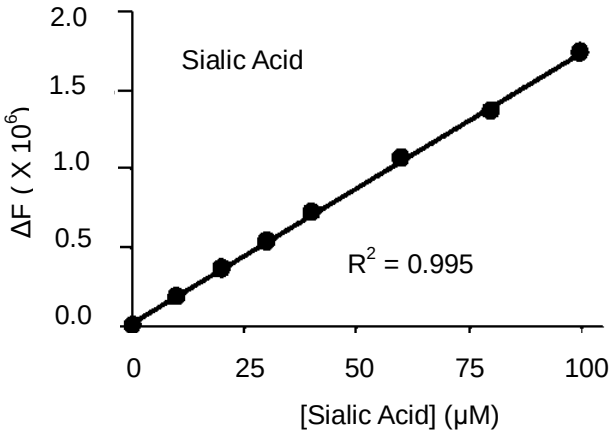
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and dH_2O Blank (#4), respectively. n is the sample dilution factor, n = 5 for TSA assays and n = 1 for FSA assays.

Note: if the Sample OD value is higher than that for the 1000 µM Standard, or 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: 1000 µM NANA equals 30.9 mg/dL or 309 ppm.



96-well colorimetric assay



96-well fluorimetric assay

Resources

References

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3. Sherblom, A.P. et al (1988). Bovine serum sialic acid: age-related changes in type and content. Int J Biochem. 20:1177-1183.