Product Manual

Total Sialic Acid Assay Kit (Fluorometric)

Catalog Number

MET-5157 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The term "Sialic Acid" is used to describe a family of derivatives of nine-carbon monosaccharides. There are at least 43 derivatives containing either N- or O- substitutions: the most common derivative is N-acetylneuraminic Acid (NANA or Neu5Ac). While millimolar amounts of sialic acid have been detected in normal human serum, the highest intracellular amounts are found in brain gangliosides which function in neurotransmission, memory storage, as well as synapse formation. Additionally, proteins rich in sialic acid, known as sialoglycoproteins, can bind the cell adhesion molecule Selectin. A buildup of these sialoglycoproteins on surface of metastatic cancer cells causes a significant increase in cell surface negative charge, causing repulsion from other cells and facilitating cancer cell entry into the bloodstream. Severe autosomal recessive neurodegenerative disorders known as sialic acid storage diseases (SSDs) are caused by a defect in transporting sialic acid across the lysosomal membrane, leading to accumulation of sialic acid in tissues, fibroblasts, and urine. This lack of transport can be detected by quantification of free sialic acid in urine. Furthermore, certain viruses and bacteria use sialic acid for host binding and recognition. For example, all strains of influenza A virus need sialic acid to connect with cells. When influenza A virus binds a sialic acid receptor on the cell surface, the cell internalizes the virus leading to cell infection.

Cell Biolabs' Total Sialic Acid Assay Kit is a simple fluorometric assay that measures the amount of total sialic acid present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, sialic acid standards, and unknown samples. Sample sialic concentrations are determined by comparison with a known sialic acid standard. The kit has a detection sensitivity limit of 6.25 µM sialic acid.

Note: Each sample replicate requires 2 assays, one treated with sialic acid aldolase (+SAA) and one without (-SAA). Sialic Acid is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Total Sialic Acid Assay Kit measures total Sialic Acid within biological samples. Sialic acid attached to proteins and other molecules is first converted to a free form by treatment with neuraminidase. Then sialic acid is converted to pyruvate in the presence of sialic acid aldolase. Finally, pyruvate is oxidized by pyruvate oxidase in the presence of phosphate and oxygen into acetyl phosphate, carbon dioxide, and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of sialic acid standard within the 96-well microtiter plate format. Samples and standards are incubated for 30 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

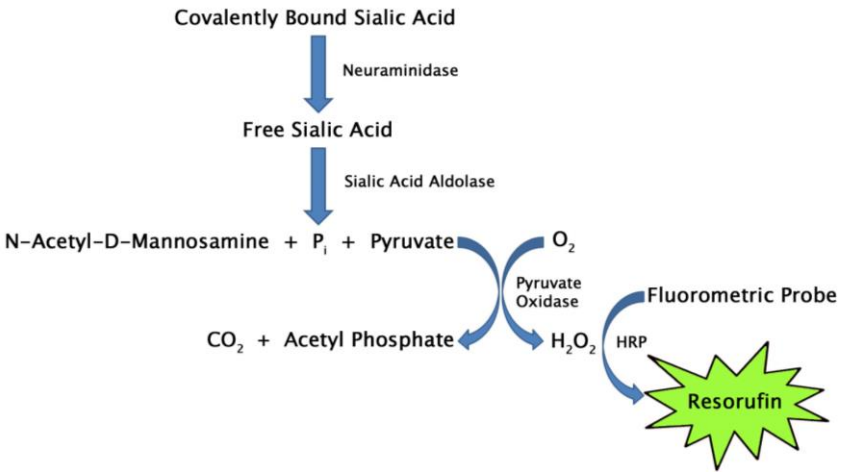

Figure 1. Sialic Acid Assay Principle.

Related Products

1. MET-5029: Pyruvate Assay Kit (Fluorometric)
2. MET-5162: Phosphoenolpyruvate Assay Kit (Fluorometric)
3. MET-5023: Glycogen Assay Kit (Fluorometric)
4. MET-5019: Total Phosphatidic Acid Assay Kit (Fluorometric)
5. MET-5159: Pyrophosphate Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped on blue ice packs)

1. Sialic Acid Standard (Part No. 50151C): One 50 µL tube of n-acetyl neuraminic acid at 160 mM.
2. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
3. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
4. FAD (Part No. 50293C): One 50 µL tube of 2 mM Flavin Adenine Dinucleotide (FAD).
5. TPP (Part No. 50294C): One 50 µL tube of 2 mM Thiamine Pyrophosphate (TPP).
6. Neuraminidase (Part No. 51562D): One 300 µL tube.
7. Sialic Acid Aldolase (Part No. 51563D): One 500 µL tube.
8. Pyruvate Oxidase (Part No. 50295C): One 300 µL tube.

Box 2 (shipped on blue ice packs)

1. 10X Assay Buffer (Part No. 50292A): One 25 mL bottle.
2. 10X Reaction Buffer (Part No. 51561A): One 1.4 mL tube.

Materials Not Supplied

1. Distilled or deionized water
2. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter (e.g., Amicon Ultra 0.5mL)

3. Phosphate Buffered Saline (PBS)
4. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate

Storage

Upon receipt, store the 10X Reaction Buffer and the 10X Assay Buffer at 4°C. Store Neuraminidase and Sialic Acid Aldolase at -80°C. Store all other components at -20ºC or -80°C. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at 4°C.
- Reaction Mix and Negative Control Mix: Prepare two separate mixtures according to the table below. The Sialic Acid Aldolase is omitted from the Negative Control Mix.

Component	Reaction Mix (20 assays)	Negative Control Mix (20 assays)
Sialic Acid Aldolase	100 µL	------
HRP	2 µL	2 µL
Pyruvate Oxidase	60 µL	60 µL
FAD	10 µL	10 µL
TPP	10 µL	10 µL
Fluorometric Probe	10 µL	10 µL
1X Assay Buffer	808 µL	908 µL
Total	**1000 µL**	**1000 µL**

Note: Prepare only enough for immediate use and scale proportionally as needed.

Preparation of Samples

- Cell culture supernatants: Cell culture media formulated with pyruvate should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Transfer 240 µL to a new tube and add 28 µL of 10X Reaction Buffer and 6 µL of Neuraminidase. Incubate at 37°C for 30 minutes. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. The deproteinated sample may be assayed directly or diluted as necessary in 1X Assay Buffer.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Recover the supernatant. Transfer 240 µL to a new tube and add 28 µL of 10X Reaction Buffer and 6 µL of Neuraminidase. Incubate at 37°C for 30 minutes. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. The deproteinated sample may be assayed directly or diluted as necessary in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris and recover the supernatant. Transfer 240 µL to a new tube and add 28 µL of 10X Reaction Buffer and 6 µL of Neuraminidase. Incubate at 37°C for 30 minutes. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. The deproteinated sample may be assayed directly or diluted as necessary in 1X Assay Buffer.

- Serum, plasma, saliva, or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min and recover the supernatant. Transfer 240 µL to a new tube and add 28 µL of 10X Reaction Buffer and 6 µL of Neuraminidase. Incubate at 37°C for 30 minutes. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. The deproteinated sample may be assayed directly or diluted as necessary in 1X Assay Buffer.

Notes:

- *Each sample prepared above must be run in separate wells with the Reaction Mix and the Negative Control Mix.*
- *If desired, the above samples can be assayed without treatment with Neuraminidase to measure only the free sialic acid in the sample.*
- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Sialic Acid standards immediately before use by thoroughly thawing the Sialic Acid Standard and diluting in 1X Assay Buffer according to Table 1 below.

Standard Tubes	160 mM Sialic Acid Standard (µL)	1X Assay Buffer (µL)	Sialic Acid (µM)
1	4	1596	400
2	300 of Tube #1	300	200
3	300 of Tube #2	300	100
4	300 of Tube #3	300	50
5	300 of Tube #4	300	25
6	300 of Tube #5	300	12.5
7	300 of Tube #6	300	6.25
8	0	300	0

Table 1. Preparation of Sialic Acid Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including neuraminidase treated unknowns (see preparation of samples section) and standards, should be assayed in duplicate or triplicate.

Note: Each unknown sample replicate requires two paired wells, one to be treated with Sialic Acid Aldolase (Reaction Mix) and one without the enzyme (Negative Control Mix) to measure endogenous background (1X Assay buffer is added to the Negative Control Mix in place of Sialic Acid Aldolase).

2. Add 50 µL of each sialic acid standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.

4. Add 50 µL of Negative Control Mix to the other half of the paired sample wells.
5. Mix the well contents thoroughly and incubate for 30 minutes at 37ºC protected from light.

Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

6. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Example of Results

The following figures demonstrate typical Total Sialic Acid Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

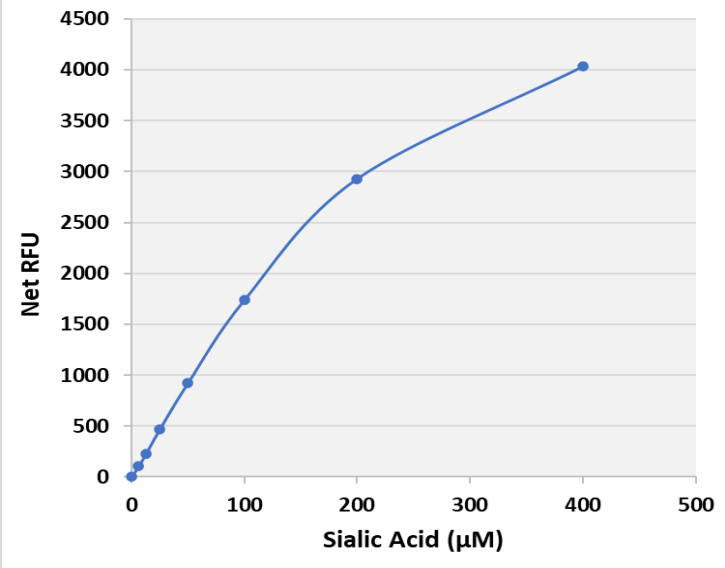

Figure 2: Sialic Acid Standard Curve.

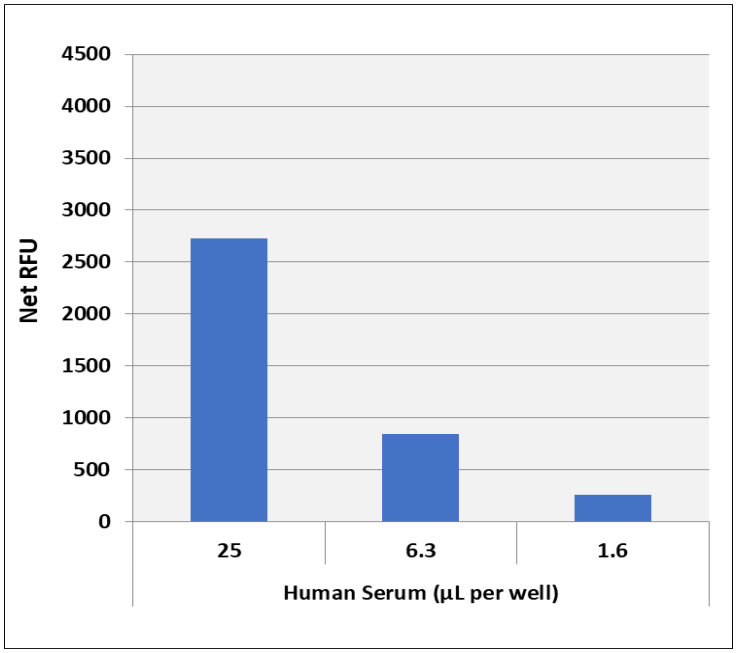

Figure 3: Sialic Acid detection in deproteinated human serum using the Total Sialic Acid Assay Kit.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Sialic Acid Aldolase (-SAA) from the sample well values containing Sialic Acid Aldolase (+SAA) to obtain the difference. The fluorescence difference is due to the Sialic Acid Aldolase activity and removes background due to endogenous pyruvate.

$$\textbf{Net RFU} = (\textbf{RFU}_{\textbf{+SAA}}) - (\textbf{RFU}_{\textbf{-SAA}})$$

5. Compare the adjusted Net RFU of each sample to the standard curve to determine and extrapolate the quantity of sialic acid present in the sample. Only use values within the range of the standard curve.

Note: If the unknown samples were treated with 10X reaction buffer and Neuraminidase, then multiply the sialic acid concentration by 1.124 to account for the volume change.

References

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Warranty

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