Sialic Acid

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1183

(Version 1.3A)

Detection and Quantification of Sialic AcidContentin Serum, Plasma, Cell culture, Saliva, Milk, Tissue extracts, Cell lysate, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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. Thesialic 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.

Sialic AcidColorimetric Microplate Assay Kit is designed to directly measure sialic acid in a variety of samples. In the assay, sialic acid reacts with Resorcinol, which is determined at 570nm, is directly proportional to the sialic acidconcentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay BufferI	30 mlx 2	4 °C
Assay BufferII	30 mlx 2	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Dye Reagent: Add 10 ml Dye Reagent Diluent to dissolve before use.Store at 4 °C and use within 24 hoursafter reconstitution.

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 100 mmol/L of standardstock solution, store at 4°C for 2-3 weeks or -20°C for3 months after reconstitution.Then dilute to1000µmol/Lstandard top solutionby adding 10 µl stock solution into 990µl distilled water.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be1000/500/250/125/62.5/31.2/15.6µmol/L.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5mlAssay Buffer I for 5×10^6 cell or bacteria, mix, incubate at 80°C water bath for 1 hour; centrifuged at 8000g for 10minutes, take the supernatant into a new centrifuge tube;then add 0.5 ml Assay Buffer II, mix, centrifuged at 8000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

2.For tissue samples

Weigh out 0.01 g tissue, homogenize with 0.5mlAssay BufferI, then transfer it into a new centrifuge tube, incubate at 80 °C water bath for 1 hour; centrifuged at 8000g for 10minutes, take the supernatant into a new centrifuge tube; then add 0.5 ml Assay Buffer II, mix, centrifuged at 8000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

3.For serum, plasma, urine and other biological fluids samples

Add 0.1 ml sample into 0.4 ml Assay Buffer II, mix, centrifuged at 8000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it into the oven,90°C for 30minutes. Then record absorbance measured at 570 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}\times V/(V+V_{Assay})}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer I and Assay Buffer II, μl

$C_{Protein}$: the sample protein concentration, mg/ml

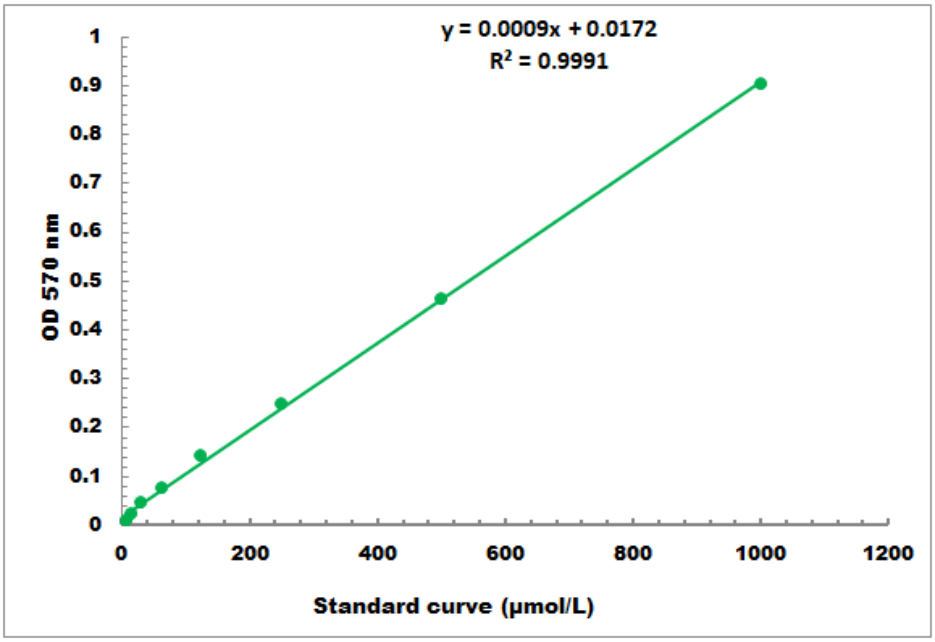
W: the weight of sample, g

V: the volume of sample in sample preparation, ml

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 10μmol/L - 1000μmol/L