Sialic Acid Microplate Assay Kit

User Manual

Catalog # CAK1183

(Version 1.2A)

Detection and Quantification of Sialic Acid content in Serum, Plasma, Cell culture, Saliva, Milk, Tissue extracts, Cell lysate, Other biological fluids Samples.

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. 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.

Sialic Acid 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 acid concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 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	
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Note:

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve, then add 10 µl into 990 µl distilled water, mix, the concentration will be 1000 µmol/L.

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. SAMPLE PREPARATION

1. For cell and bacteria samples

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

2. For tissue samples

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

V. ASSAY PROCEDURE

Add following reagents into the 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 30 minutes. Then record absorbance measured at 570 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$SA\ (\mu mol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times 5$$

$$= 5000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= 1000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 1000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the standard concentration, 1000 μmol/L;

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

W: the weight of sample, g;

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

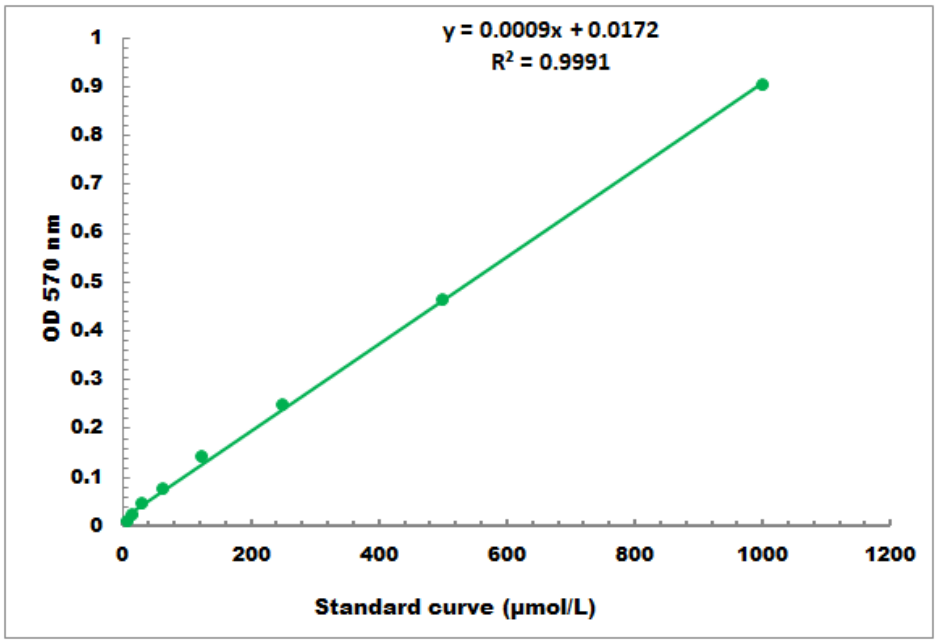
V_{Assay}: the volume of Assay buffer I and Assay buffer II, 1 ml

$V_{Standard}$: the volume of standard, 100 μl;

V_{Sample}: the volume of sample, 100 μl.

VII. TYPICAL DATA

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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES