(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Sialic Acid (SA) Assay Kit

Catalog No.: BC00037

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Sialic Acid (SA) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, Serum, Plasma
Assay Type	Quantitative
Detection Instrument	Microplate reader (450 nm)

Product Introduction

Sialic acid is a negatively charged ion that makes saliva feel smooth. It not only has the function of "inducing" invading bacteria, but also is a transmitter of gangliosides and a component of the brain.

Principle

Sialic acid (SA) forms a purple-red complex with resorcinol under acidic conditions , and the absorbance conforms to the Lambert-Beer law. By measuring the absorbance of the complex and the standard, the content of sialic acid can be calculated. When this kit detects tissue samples, the total protein concentration needs to be measured. It is recommended to use BCA Protein Assay Kit (BCA Method)(BC00006) produced by EnkiLife.

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Chromogen A	5ml/bottle	-20℃, store at -20℃ away from light after opening, valid for 6 months.
Reagent 2	Chromogen B	20ml/bottle	-20℃, store at 2-8℃ away from light after opening, valid for 6 months.
Reagent 3	Chromogen C	30ml/bottle	-20℃, store at 2-8℃ away from light after opening, valid for 6 months.
Reagent 4	8mmol/L Sialic Acid Standard	1ml/tube, 2 tubes	-20℃, store at 2-8℃ away from light after opening, valid for 6 months.
Reagent 5	Protein Precipitants	10ml	-20℃, store at 2-8℃ after opening, valid for 6 months.
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.

Experimental Preparation

● Sample processing

1. Sample processing

Liquid samples such as serum and plasma: Generally, they can be measured directly. If the absorbance exceeds the detection range, they can be diluted to an appropriate multiple for testing.

Tissue samples: Take fresh tissue blocks, rinse with 2-8℃ deionized water, absorb with filter paper, weigh, put into homogenizer, and perform conventional homogenization (normal saline (0.9% NaCl solution) or PBS (0.01M, pH=7.4) at a ratio of weight (g): volume (mL) = 1:9. After homogenization, centrifuge at 4℃, 10000×g for 10 min, take the supernatant and place on ice for testing. Keep part of the supernatant for protein concentration determination.

2. Dilution of samples

Before formal testing, it is necessary to select 2-3 samples with large expected differences and dilute them into different concentrations for preliminary experiments, and determine the actual dilution multiple of the samples based on the results of the preliminary experiments.

Note: The diluent is physiological saline (0.9% NaCl) or PBS (0.01 M, pH7.4).

- Preparation of the assay kit

1. Reagent 4 was taken out from -20℃ and slowly thawed on ice (it is best to divide it into smaller portions to avoid repeated freezing and thawing), and the other reagents were equilibrated to room temperature.

2. Preparation of chromogen: Mix reagent 1: reagent 2: reagent 3 in a volume ratio of 1:10:160, and dilute to 1.17 times the original volume with double distilled water. Prepare before use and store at 2-8℃.

3. Dilution of standards of different concentrations: 8 mmol/L sialic acid standard was diluted to different concentrations: 8, 4, 2, 1, 0.5, 0 mmol/L using double distilled water by half dilution method.

Key points of the experiment

1. 100 ℃ water bath time must be sufficient and the boiling water level must be higher than the reagent level in the EP tube.
2. After centrifugation, take the supernatant and be careful not to transfer the precipitate

into the ELISA plate.

Operation process

1. Preparation of supernatant: Mix the sample and protein precipitant at a volume ratio of 1:1 (for example, take 20μL of sample into a 1.5mL EP tube, add 20μL of protein precipitant and mix), centrifuge at 1100×g for 10 min, and take the supernatant for testing.
2. Take 25 μL of standards of different concentrations and supernatant and add them into the corresponding 1.5mL EP tubes.
3. Add 500 μL of colorimetric reagent to each tube in step (2).
4. The tubes were placed in a boiling water bath for 15 minutes and then in an ice water bath for 3 minutes.
5. Take 200 μL of solution from the corresponding EP tube and add it to the corresponding standard well and measurement well.
6. The OD value of each well was measured using a microplate at 450nm.

Operation table (operation in EP tube)

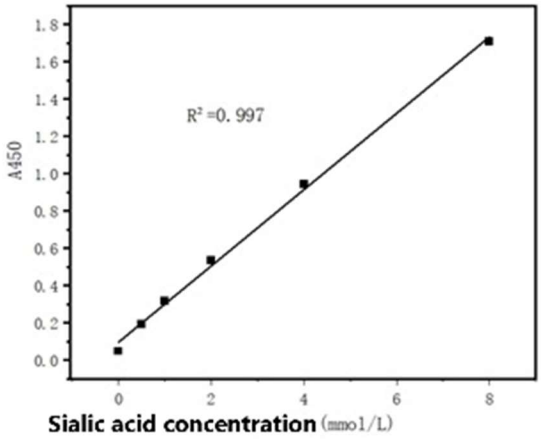
	Standard tube	Assay tube	Blank tube
Different concentrations of standard products (μL)	25	--	--
Double distilled water (μL)	--	--	25
Sample to be tested (μL)	--	25	--
Chromogen (μL)	500	500	500
After boiling in water for 15 minutes, place in ice water for 3 minutes, take 200 μL of the solution to the ELISA plate, and measure the OD value at 450 nm using an ELISA reader.			

Result calculation

Standard fitting curve: y = ax + b
The calculation formula of sialic acid content in serum (plasma) and milk is:
Sialic acid content (mmol/L) **=** (ΔA 450 - b) ÷ a × f
The calculation formula of sialic acid content in tissue is:
Sialic acid content (mmol/gprot) **=** (ΔA 450 - b) ÷ a ÷ Cpr × f
Annotation:
y: OD value of standard well-OD value of blank well (OD value when the concentration of standard is 0)
x: concentration of the standard
a: Slope of the standard curve
b: standard curve intercept
ΔA 450: Sample measurement OD value - blank OD value (OD value when the standard concentration is 0)
f: dilution factor of the sample before adding it to the detection system

Cpr: protein concentration of the sample to be tested (gprot/L)
The following standard curve is for reference

Sialic acid concentration (mmol/L)	A450
8	1.708
4	0.943
2	0.536
1	0.316
0.5	0.193
0	0.048



$y=0.2046X+0.0955(R^2=0.997)$

Notes

1. The kit is for Research Use Only. If it is used for clinical diagnosis or any other purpose,our company will not be responsible for any problems arising therefrom and will not bear any legal liability.

2. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.

3. Please wear lab coats and latex gloves for protection during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.

5. If the sample being tested is not among the sample types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.

6. The final experimental results are closely related to the effectiveness of the reagents, the relevant operations of the experimenter, the experimental environment and other factors. Our company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible usage of the sample before use and reserve sufficient samples.