Sialic Acid (SA) Colorimetric Assay Kit

Catalog No: E-BC-K068-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.022 mmol/L

Detection range: 0.022-7 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the Sialic Acid (SA) content in serum, plasma, tissue, saliva, urine and hydrothorax samples.

▲Background

Sialic acid is a general name of derivatives with nine carbon glycosylneuraminic acid. Sialic acid is widely distributed in animal tissues, mainly in glycoprotein and ganglioside. The main function of sialic acid is to participate in various recognition processes between cells and molecules. N-acetyl neuraminic acid, an important molecule in biometrics, is the representative of sialic acid family.

▲Detection principle

Sialic acid forms a purplish red complex with methyl resorcinol in the presence of oxidant. And the absorbance conforms to Lambert-Beer's law. The content of sialic acid can be calculated by measuring the OD value at 560 nm.

When detect tissue and cell samples, the protein concentration of the sample needs to be detected in addition (E-BC-K165, E-BC-K168, E-BC-K318 are recommended).

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	1 mmol/L SA Standard	1 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 2	Chromogenic Agent	60 mL × 8 vials	2-8℃ , 6 months, shading light
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (560 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 10 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The incubation time of water bath at 100°C should be sufficient (15 min), and the level of water bath liquid should be higher than the reagent in the test tube.
2. When measuring the OD value, the sediment shouldn't be added into the cuvette, the pipette is recommended.

Pre-assay preparation

▲Reagent preparation

Take the reagent 1 from -20 °C and place on ice to thaw slowly. It is recommended to aliquot the reagent 1 to avoid repeated freezing and thawing.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.022-7 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Rat plasma	1
10% Carrot tissue homogenization	1
Human saliva	1
10% Mouse liver tissue homogenization	1
10% Mouse brain tissue homogenization	1
Human hydrothorax	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	0

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Blank tube: For serum (plasma), saliva and other liquid samples, add 0.1 mL double distilled water into a 5 mL EP tube. For tissue sample, add 0.2 mL double distilled water into a 5 mL EP tube

   Standard tube: For serum (plasma), saliva and other liquid samples, add 0.1 mL of 1 mmol/L SA standard into a 5 mL EP tube. For tissue sample, add 0.1 mL of 1 mmol/L SA standard and 0.1 mL of double distilled water into a 5 mL EP tube.

   Sample tube: For serum (plasma), saliva and other liquid samples, add 0.1 mL sample into a 5 mL EP tube. For tissue sample, add 0.2 mL sample into a 5 mL EP tube.

2. Add 4 mL of reagent 2 into each tube.
3. Mix fully and fasten the mouth of the tube with plastic film, prick a small hole with a needle. Incubate the tubes at 100℃ for 15 min.
4. Take out the tubes and cool with running water. Centrifuge at 2325 g for 10 min.
5. Set the spectrophotometry to zero with ddH_2O, take the supernatant and measure the OD values of each tube at 560 nm with 1 cm optical path cuvette.

▲Operation table

For serum (plasma), saliva and other liquid sample:

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.1		
1 mmol/L SA standard (mL)		0.1	
Sample (mL)			0.1
Reagent 2 (mL)	4.0	4.0	4.0
Mix fully with a vortex mixer, then fasten the mouth of the tube with plastic film, prick a small hole with a needle. Incubate the tubes at 100°C for 15 min. Take out the tubes and cool with running water. Centrifuge at 2325 g for 10 min. Set the spectrophotometry to zero with ddH_2O. Take the supernatant and measure the OD values of each tube at 560 nm with 1 cm optical path cuvette.			

For tissue and cells sample:

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.2	0.1	
1 mmol/L SA standard (mL)		0.1	
Sample (mL)			0.2
Reagent 2 (mL)	4.0	4.0	4.0
Mix fully with a vortex mixer, then fasten the mouth of the tube with plastic film, prick a small hole with a needle. Incubate the tubes at 100°C for 15 min. Take out the tubes and cool with running water. Centrifuge at 2325 g for 10 min. Set the spectrophotometry to zero with ddH_2O. Take the supernatant and measure the OD values of each tube at 560 nm with 1 cm optical path cuvette.			

▲Calculation

Serum (plasma) and other liquid sample:

$$\text{SA content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f$$

Tissue and cells sample:

$$\text{SA content (mmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c_1: Concentration of standard, 1 mmol/L

c_2: Concentration of standard, 0.5 mmol/L. For tissue and cells sample, the volume of standard in operation step is 0.2 mL (0.1 mL of 1 mmol/L SA Standard + 0.1 mL of distilled water), so the concentration of Standard is 0.5 mmol/L

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, gprot/L

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.022-7 mmol/L	Average intra-assay CV (%)	3.9
Sensitivity	0.022 mmol/L	Average inter-assay CV (%)	7.1
Average recovery rate (%)	95		

▲Example analysis

Take 0.1 mL of rat plasma, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.252, the average OD value of the blank is 0.003, the average OD value of the standard is 0.066, the concentration of standard is 1 mmol/L, and the calculation result is:

$$\text{SA content (mmol/L)} = \frac{0.252-0.003}{0.066-0.003} \times 1\text{mmol/L} = 3.95\ \text{mmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Saliva

Saliva was collected 30 min after gargling with clear water and centrifuged at 10000 g at 4℃ for 5 min. Then take the supernatant for detection. If not detected on the same day, the saliva can be stored at -80℃ for a month.

▲Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲Hydrothorax

Take fresh hydrothorax into the tube which contain anticoagulant (EDTA, 0.1 mg/mL). Centrifuge at 10000 g for 10 min at 4°C . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the hydrothorax can be stored at -80°C for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8°C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 3100 g at 4°C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

▲Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^5): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at-80°C for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.