PRODUCT INFORMATION & MANUAL

Sialic Acid Assay Kit (Colorimetric)

NBP3-24534

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Sialic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-24534

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.03 mmol/L

Detection range: 0.03-7 mmol/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 6.5

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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 biometric systems, 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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	8 mmol/L SA Standard	1 mL × 2 vials	-20℃, 12 months, shading light
Reagent 2	Chromogenic Agent	30 mL × 2 vials	2-8℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (510-570 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

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 level of water bath should be higher than the level of liquid in the EP tube when incubation. And the time of 100℃ incubation should be sufficient.
2. Take the supernatant carefully after centrifugation, do not transfer the precipitate into the microplate.

Pre-assay preparation

▲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 (0.03-7 mmol/L).

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

Sample type	Dilution factor
Human serum	1
Rat serum	1
Mouse serum	1
Porcine serum	1
Human plasma	1
Rat plasma	1
Mouse plasma	1
Human saliva	1
Human urine	1
Human hydrothorax	1
10% Plant tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse brain tissue homogenate	1

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

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 8 mmol/L SA standard with double distilled water to a serial concentration.

For serum (plasma), saliva and other liquid sample, the recommended dilution gradient is 0, 1, 2, 3, 4, 5, 6, 7 mmol/L.

For tissue sample, the recommended dilution gradient is 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	8 mmol/L SA standard (μL)	Double distilled water (μL)
A	0	0	200
B	1	25	175
C	2	50	150
D	3	75	125
E	4	100	100
F	5	125	75
G	6	150	50
H	7	175	25

The measurement of samples

1. Standard tube: For serum (plasma), saliva and other liquid sample, add 25 μL of standard with different concentrations into a 2 mL EP tube. For tissue sample, add 25 μL of double distilled water and 25 μL of standard with different concentrations into a 2 mL EP tube.
   Sample tube: For serum (plasma), saliva and other liquid sample, add 25 μL of sample into a 2 mL EP tube. For tissue sample, add 50 μL of sample into a 5 mL EP tube.
2. Add 500 μL of reagent 2 into each tube.
3. Mix fully with a vortex mixer, incubate the tubes at 100℃ with water bath for 15 min (The level of water bath is higher than the level of liquid in the EP tube).
4. Take out the tubes and cool with running water. Centrifuge at 2325 g for 10 min.
5. Take 200 μL of the supernatant to microplate (Do not transfer the precipitate to the plate) and measure the OD values of each well at 560 nm with microplate reader.

▲Summary operation table

For serum (plasma), tissue and cells samples

	Standard tube	Sample tube
Standard with different concentrations (μL)	25	
Sample (μL)		25
Reagent 2 (μL)	500	500
Mix fully, incubate the tubes at 100℃ for 15 min. Take out the tubes and cool. Centrifuge at 2325 g for 10 min. Take 200 μL of the supernatant to microplate and measure the OD values of each well.		

For tissue sample

	Standard tube	Sample tube
Double distilled water (μL)	25	
Standard with different concentrations (μL)	25	
Sample (μL)		50
Reagent 2 (μL)	500	500
Mix fully, incubate the tubes at 100℃ for 15 min. Take out the tubes and cool. Centrifuge at 2325 g for 10 min. Take 200 μL of the supernatant to microplate and measure the OD values of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: y= ax + b.

y: The absolute OD value of standard;

x: The concentration of Standard;

a: The slope of standard curve;

b: The intercept of standard curve.

1. For serum (plasma), saliva and other liquid samples

The standard curve is: $y= a_1x + b_1$.

$$\text{SA content (mmol/L)} = (\Delta A_{560} - b_1) \div a_1 \times f$$

2. For tissue samples

The standard curve is: $y= a_2x + b_2$.

$$\text{SA content (mmol/gprot)} = (\Delta A_{560} - b_2) \div a_2 \div C_{pr} \times f$$

Note:

ΔA_{560}: Absolute OD (OD_{Sample} - OD_{Blank})

f: the dilution multiple of tested samples.

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

Appendix I Data

▲Example analysis

Take 25 μL of mouse plasma and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0817 x – 0.0069, the average OD value of the sample is 0.194, the average OD value of the blank is 0.042, and the calculation result is:

$$\text{SA content (mmol/L)} = \frac{0.194 - 0.042 + 0.0069}{0.0817} = 1.94 \text{ (mmol/L)}$$

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

30 min after gargled, collect the fresh saliva sample, centrifuge at 10000 g for 5 min at 4℃ , take the supernatant to preserve it on ice for detection. If not detected on the same day, the plasma 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

Collect the fresh hydrothorax to the tubes with anticoagulant and mix fully. Centrifuge the sample at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

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

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.)

Appendix III References

1. Traving C, Schauer R. Structure, function and metabolism of sialic acids[J]. Cellular & Molecular Life Sciences Cmls, 1998, 54(12): 1330-1349.
2. Maru I, Ohnishi J, Ohta Y, et al. Why is sialic acid attracting interest now? Complete enzymatic synthesis of sialic acid with N-acylglucosamine 2-epimerase[J]. Journal of Bioscience & Bioengineering, 2002, 93(3): 258-265.