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

Catalog No：E-BC-K068-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（510-570 nm）

Detection range：0.03-7 mmol/L

Elabscience®Sialic Acid (SA) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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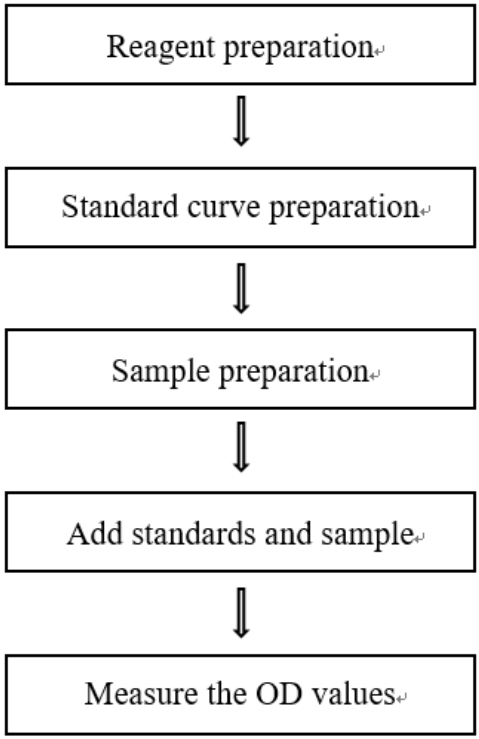
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	8 mmol/L SA Standard	1 mL ×1 vial	1 mL ×2 vials	-20℃, 12 months, shading light
Reagent 2	Chromogenic Agent	30 mL ×1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Keep 8 mmol/L SA standard on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
8 mmol/L SA standard (μL)	0	25	50	75	100	125	150	175
Double distilled water (μL)	200	175	150	125	100	75	50	25

Sample preparation

① Sample preparation

Serum, plasma and saliva: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

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.

Operating steps

For serum (plasma), saliva and other liquid sample

① Standard tube: add 25 μL of standard with different concentrations into a 2 mL EP tube.

Sample tube: add 25 μL of sample into a 2 mL EP tube.

② Add 500 μL of chromogenic agent 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.

For tissue sample

① Standard tube: add 25 μL of double distilled water and 25 μL of standard with different concentrations into a 2 mL EP tube.

Sample tube: add 50 μL of sample into a 5 mL EP tube.

② Add 500 μL of chromogenic agent 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma), saliva and other liquid samples:

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

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

2. Tissue sample:

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

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

[Note]

ΔA560: Absolute OD ($OD_{Sample} - OD_{Blank}$)

f: the dilution multiple of tested samples.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L）	1.30	4.60	5.80
%CV	2.5	2.0	2.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.30	4.60	5.80
%CV	6.5	6.3	6.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.5	3.8	5.4
Observed Conc. (mmol/L)	1.5	3.9	5.5
recovery rate(%)	101	103	102

Sensitivity

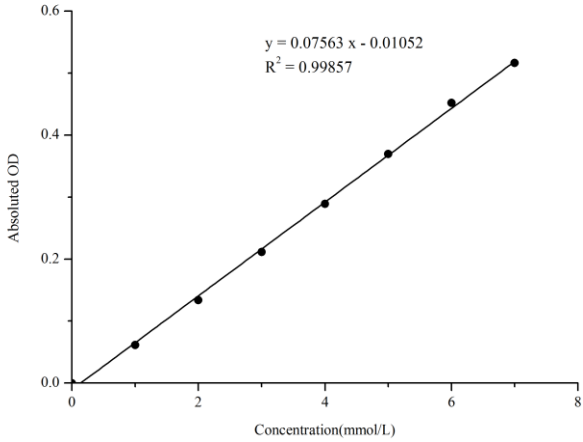
The analytical sensitivity of the assay is 0.03 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard

was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Average OD	0.043	0.104	0.177	0.255	0.332	0.413	0.495	0.559
Absoluted OD	0	0.061	0.134	0.212	0.289	0.370	0.452	0.516



Appendix Π Example Analysis

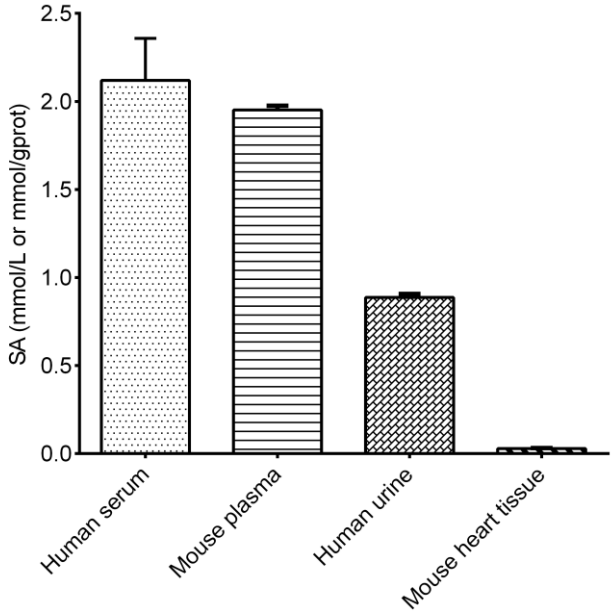
Example analysis：

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:

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

Detect human serum, mouse plasma, human urine, 10% mouse heart tissue homogenate (the concentration of protein is 7.15 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.