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

Catalog No：E-BC-K068-S

Specification：50 assays(48 samples)/ 100 assays(96 samples)

Measuring instrument：Spectrophotometer（560 nm）

Detection range：0.022-7 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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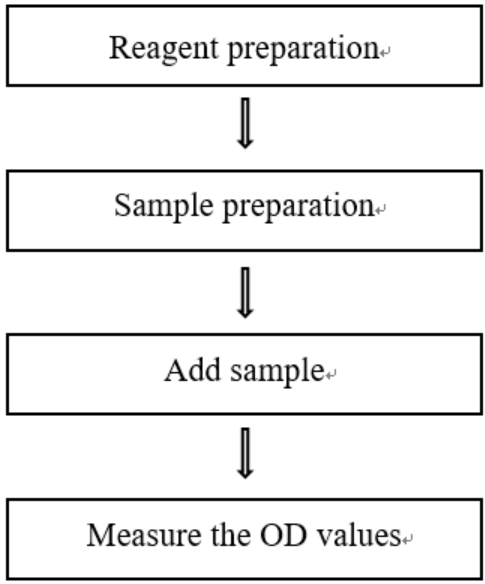
Assay summary



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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	1 mmol/L SA Standard	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Chromogenic Agent	60 mL × 4 vials	60 mL × 8 vials	2-8℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

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

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 30 mg).

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

③ Homogenize 30 mg tissue in 270 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
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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The incubation time of water bath at 100℃ 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.

Operating steps

For serum (plasma), saliva and other liquid sample

① Blank tube: add 0.1 mL double distilled water into 5 mL EP tube
Standard tube: add 0.1 mL of 1 mmol/L SA standard into 5 mL EP tube.
Sample tube: add 0.1 mL sample into 5 mL EP tube.

② Add 4 mL of chromogenic agent 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 double distilled water, take the supernatant and measure the OD values of each tube at 560 nm with 1 cm optical path cuvette.

For tissue sample

① Blank tube: add 0.2 mL double distilled water into 5 mL EP tube
Standard tube: add 0.1 mL of 1 mmol/L SA standard and 0.1 mL of double distilled water into 5 mL EP tube.
Sample tube: add 0.2 mL sample into 5 mL EP tube.

② Add 4 mL of chromogenic agent 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 double distilled water, take the supernatant and measure the OD values of each tube at 560 nm with 1 cm optical path cuvette.

Calculation

The sample:

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

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

2. 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.

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）	0.85	3.40	6.30
%CV	4.3	4.0	3.4

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）	0.85	3.40	6.30
%CV	6.9	7.0	7.4

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 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	8	18	20
Observed Conc. (mmol/L)	7.4	17.6	19.0
recovery rate(%)	92	98	95

Sensitivity

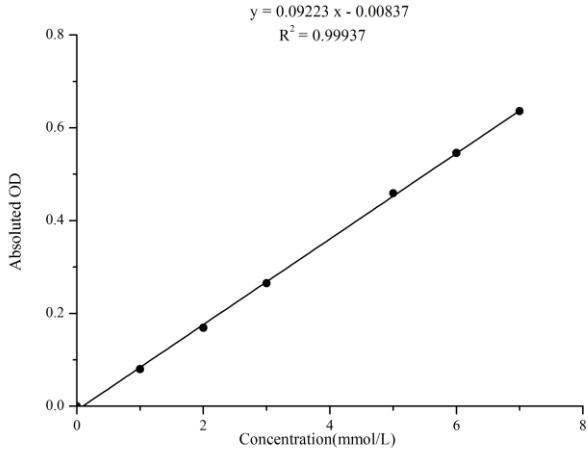
The analytical sensitivity of the assay is 0.022 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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**	**5.0**	**6.0**	**7.0**
Average OD	0.001	0.081	0.170	0.266	0.460	0.547	0.637
Absoluted OD	0	0.080	0.169	0.265	0.459	0.546	0.636



Appendix II Example Analysis

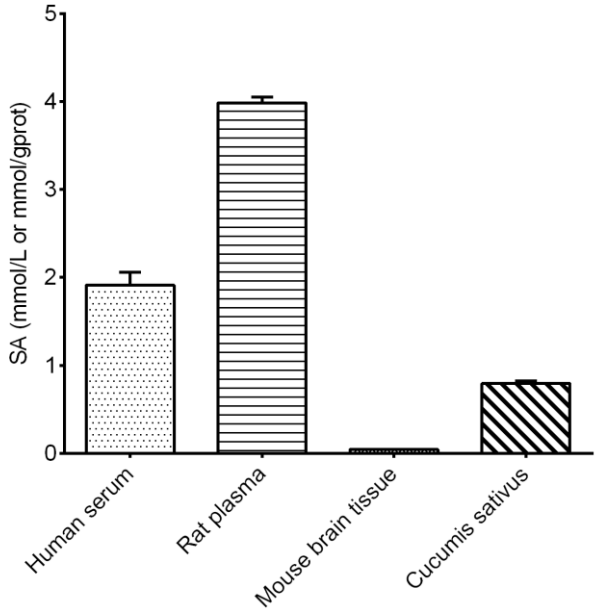
Example analysis:

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:

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

Detect human serum (V=100 μL), rat plasma (V=100 μL), 5% mouse brain tissue homogenate (the concentration of protein in sample is 2.64 gprot/L, V=200 μL), 10% cucumis sativus tissue homogenate (the concentration of protein in sample is 0.59 gprot/L, V=200 μL) 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.