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

Catalog No：E-BC-K813-M

Specification：48T (44 samples)/96T (92 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：0.05-4.00 mmol/L

Elabscience® Sialic Acid (SA) Colorimetric Assay Kit (Enzyme Method)

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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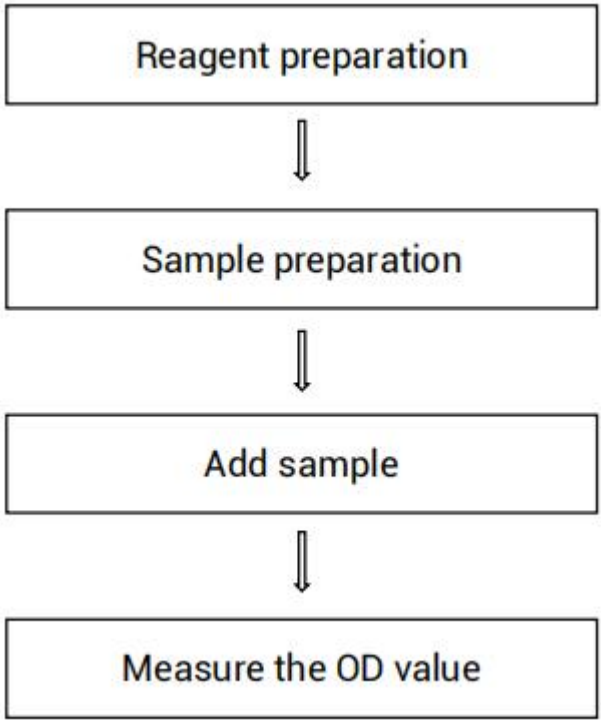
Assay summary



Intended use

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

Detection principle

Sialic acid (SA) is widely present in animals and microorganisms, usually in the form of oligosaccharides, glycolipids or glycoproteins, and is an important component of cell membranes. SA is located at the ends of cell membrane glycoproteins and glycolipids, and participates in various physiological functions such as cell recognition, adhesion and contact inhibition. It is a broad-spectrum tumor marker. When normal cells transform into malignant tumor cells, the structure and content of glycoproteins and glycolipids on the cell surface undergo significant changes. SA falls off and enters the blood, increasing the content of SA in serum. Measuring its level is of great significance for the auxiliary diagnosis, therapeutic effect observation and prognosis judgment of malignant tumors.

SA is converted into pyruvic acid under the action of a series of enzymes. Pyruvic acid is converted into lactic acid and NAD^+ under the action of lactate dehydrogenase and NADH. The concentration of SA in the sample can be indirectly measured by measuring the decrease in the absorbance of NADH at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Regent A	9 mL × 1 vial	18 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Enzyme Regent B	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	2 mmol/L Standard	0.2 mL × 1 vial	0.2 mL × 1 vial	2-8°C, 12 months, shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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 (340 nm), Incubator

Reagent preparation

Equilibrate all the reagents to 25℃ before use.

Sample preparation

① Sample preparation

Serum (plasma) and other liquid samples: detect directly.

② Dilution of sample

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

Sample type	Dilution factor
Human serum (plasma)	1
Mouse serum (plasma)	1
Rat serum (plasma)	1
Human milk	1
Human saliva	1
Rabbit serum	1
Chicken serum	1
Porcine serum	1
Horse serum	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The initial reaction rate is very fast after adding enzyme regent B. In order to ensure the accuracy of A_1 measurement, it's better to measure no more than 8 sample wells at same time (excluding blank and standard Wells).

② When measuring low content samples, the volume of sample should be increased to 10-15 μL, and the volume of blank well and standard well should be increased at the same time.

Operating steps

① Blank well: Add 7 μL of double distilled water to the corresponding well.

Standard well: Add 7 μL of 2 mmol/L standard to the corresponding well.

Sample well: Add 7 μL of samples to the corresponding well.

② Add 150 μL of enzyme regent A to each well.

③ Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min.

④ Add 50 μL of enzyme regent B to each well.

⑤ Measure the OD value of each well at 340 nm with microplate reader, as A_1.

⑥ Incubate at 37℃ for 10 min. Measure the OD value of each well at 340 nm with microplate reader, as A_2.

Calculation

The sample:

Serum (plasma) and other liquid samples:

$$\text{SA content (mmol/L)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard}} \times c \times f$$

[Note]

ΔA_{sample}: $A_{1(sample)} - A_{2(sample)}$.

ΔA_{blank}: $A_{1(blank)} - A_{2(blank)}$.

$\Delta A_{standard}$: $A_{2(blank)} - A_{2(standard)}$.

c: Concentration of standard, 2 mmol/L.

f: Dilution factor of sample before test.

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)	0.5	1.5	2.5
%CV	4.5	3.2	4.3

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)	0.5	1.5	2.5
%CV	7.3	7.6	6.3

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.5	1.5	2.5
Observed Conc. (mmol/L)	0.48	1.47	2.50
Recovery rate (%)	96	98	100

Sensitivity

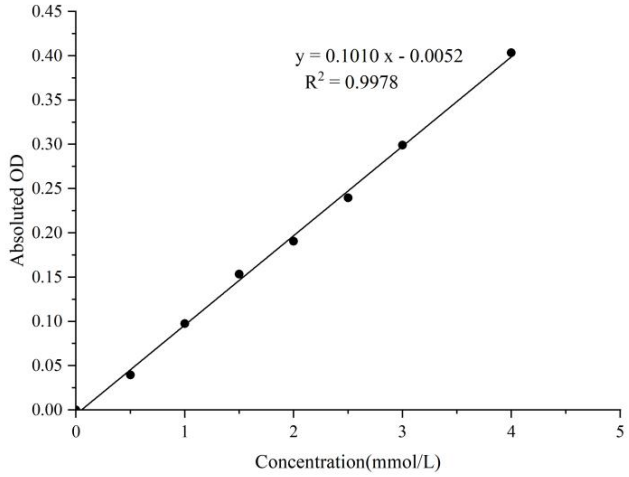
The analytical sensitivity of the assay is 0.05 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

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**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**
A_2 value	0.802	0.762	0.707	0.650	0.619	0.563	0.499	0.403
	0.804	0.765	0.704	0.649	0.606	0.564	0.509	0.396
Average A_2 value	0.803	0.764	0.706	0.650	0.612	0.564	0.504	0.400
Absoluted A_2 value	0.000	0.040	0.098	0.154	0.191	0.240	0.299	0.404



Appendix Π Example Analysis

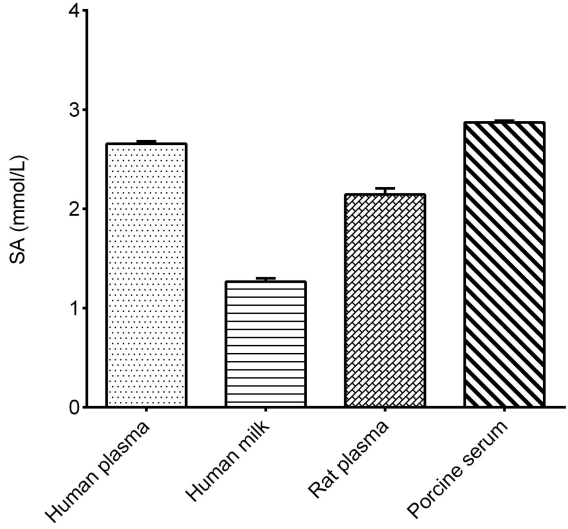
Example analysis：

Take 7 μL of human serum, and carry the assay according to the operation steps. The results are as follows:

The A_1 value of the sample well is 0.835, the A_2 value of the sample well is 0.570, ΔA_{sample} = 0.835 - 0.570 = 0.265. The A_1 value of the blank well is 0.805, the A_2 value of the blank well is 0.803, ΔA_{blank} = 0.805 - 0.803 = 0.002; the A_2 value of the standard well is 0.611, and the calculation result is:

SA content (mmol/L) = (0.265 - 0.002) ÷ (0.803 - 0.611) × 2 = 2.74 mmol/L

Detect human plamsa, human milk, rat plasma and porcine serum,



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.