TECHNICAL MANUAL

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

- **SKU CODE:** MAES0520
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add enzyme reagent A to wells
- Incubate at 37°C for 10 min
- Add enzyme reagent B to wells
- Measure absorbance at 340 nm

2. Intended use

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

3. 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.

4. Kit components & storage

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

5. Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

6. Reagent preparation

Equilibrate all reagents to 25°C before use.

7. 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): 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The initial reaction rate is very fast after adding enzyme reagent B. To ensure the accuracy of A1 measurement, it is better to measure no more than 8 sample wells at the 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.

9. 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 reagent A to each well.
3. Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min.
4. Add 50 µL of enzyme reagent B to each well.
5. Measure the OD value of each well at 340 nm with microplate reader, as A1.
6. Incubate at 37°C for 10 min. Measure the OD value of each well at 340 nm with microplate reader, as A2.

10. Calculation

The sample:

Serum (plasma) and other liquid samples:

SA content (mmol/L) = (ΔA_sample - ΔA_blank) / ΔA_standard × c × f

[Note]

ΔA_sample: A1(sample) - A2(sample).

ΔA_blank: A1(blank) - A2(blank).

ΔA_standard: A2(blank) - A2(standard).

c: Concentration of standard, 2 mmol/L.

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Results

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.

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.5	1.5	2.5
%CV	7.3	7.6	6.3

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery Results

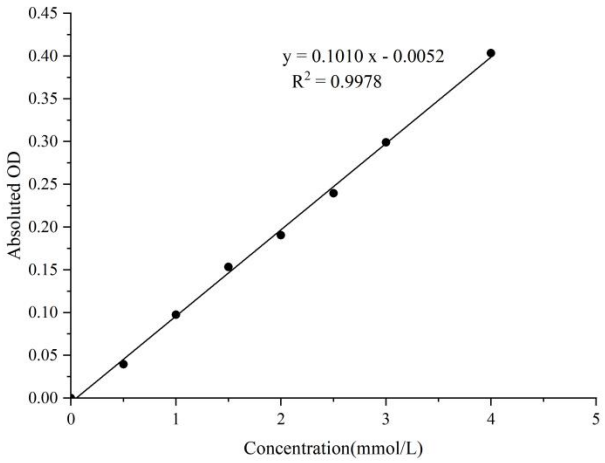
Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.5	1.5
Observed Conc. (mmol/L)	0.48	1.47
Recovery rate (%)	96	98

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.

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), the standard curve and data are provided below for reference only.



Standard curve data

Concentration (mmol/L)	A2 value 1	A2 value 2	Average A2 value	Absolute A2 value
0	0.802	0.804	0.803	0.000
0.5	0.762	0.765	0.764	0.040
1	0.707	0.704	0.706	0.098
1.5	0.650	0.649	0.650	0.154
2	0.619	0.606	0.612	0.191
2.5	0.563	0.564	0.564	0.240
3	0.499	0.509	0.504	0.299
4	0.403	0.396	0.400	0.404

12. Appendix II Example Analysis

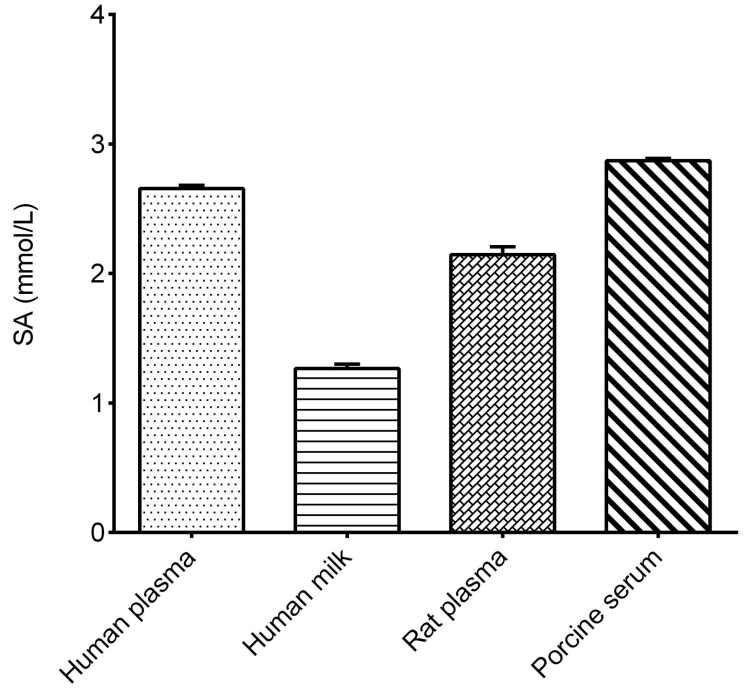
Example analysis:

Take 7 μL of human serum, and carry out 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

Detection of human plasma, human milk, rat plasma, and porcine serum according to the protocol shows the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.