TECHNICAL MANUAL

Sialic Acid (SA) Colorimetric Assay Kit

- **SKU CODE:** MAES0070
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubation at 100°C
- Centrifugation
- Measure optical density at 560 nm

2. Intended use

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

3. Detection principle

Sialic acid forms a purplish red complex with methyl resorcinol in the presence of oxidant. The absorbance conforms to Lambert-Beer's law. The content of sialic acid can be calculated by measuring the OD value at 560 nm.

4. 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°C, 12 months, shading light
Reagent 2	Chromogenic Agent	30 mL × 1 vial	30 mL × 2 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

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

6. Reagent preparation

1. Keep 8 mmol/L SA standard on ice during use. Equilibrate other reagents to room temperature before use.
2. **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 samples, the recommended dilution gradient is 0, 1, 2, 3, 4, 5, 6, 7 mmol/L. For tissue samples, the recommended dilution gradient is 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mmol/L. Reference dilution table: Item: ①②③④⑤⑥⑦⑧ 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

7. Sample preparation

Sample preparation:

Serum, plasma and saliva: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one 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°C.

④ Centrifuge at 10,000 × 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 (MAES0177).

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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The level of water bath should be higher than the level of liquid in the EP tube during incubation. The time of 100°C incubation should be sufficient.
2. Take the supernatant carefully after centrifugation. Do not transfer the precipitate into the microplate.

9. Operating steps

For serum (plasma), saliva and other liquid samples:

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°C in a water bath for 15 minutes (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 2,325 × g for 10 minutes.

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 samples:

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°C in a water bath for 15 minutes (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 2,325 × g for 10 minutes.

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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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: $y = a_1x + b_1$.

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

2. Tissue sample:

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

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.

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

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.

Inter-assay Precision

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

Recovery

Three samples of high, middle, and low concentration were tested with 6 parallel measurements each to achieve an average recovery rate of 102%.

Recovery

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.5	3.8	5.4

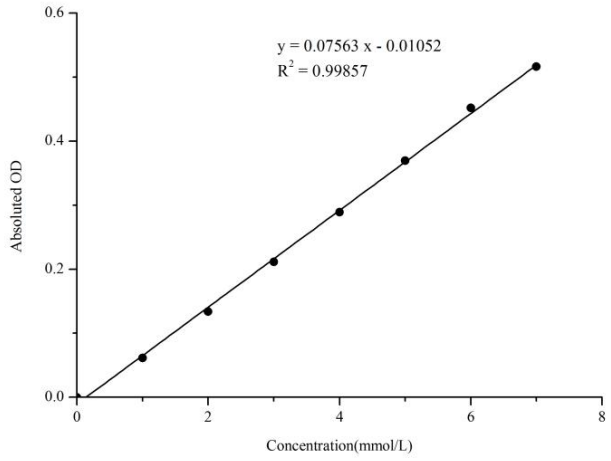
	Standard 1	Standard 2	Standard 3
Observed Conc. (mmol/L)	1.5	3.9	5.5
Recovery rate (%)	101	103	102

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.

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

Concentration (mmol/L)	Average OD	Absolute OD
0	0.043	0
1.0	0.104	0.061
2.0	0.177	0.134
3.0	0.255	0.212

Concentration (mmol/L)	Average OD	Absolute OD
4.0	0.332	0.289
5.0	0.413	0.370
6.0	0.495	0.452
7.0	0.559	0.516

12. Appendix II Example Analysis

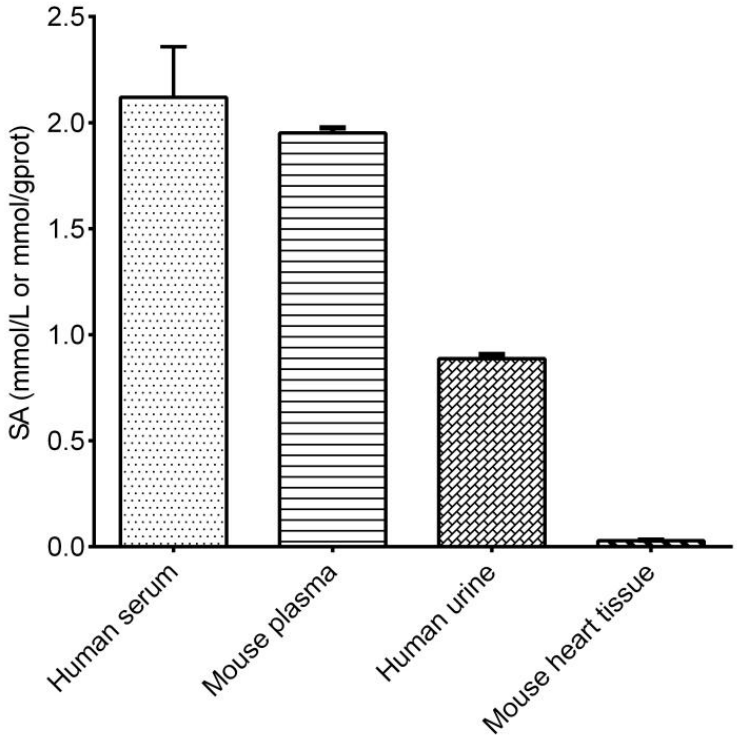
Example analysis:

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

Standard curve: y = 0.0817x - 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:

SA content (mmol/L) = (0.194 - 0.042 + 0.0069) ÷ 0.0817 = 1.94 mmol/L

Detection of human serum, mouse plasma, human urine, and 10% mouse heart tissue homogenate (the concentration of protein is 7.15 gprot/L) 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.