Sialic Acid (SA) Assay Kit

Catalog No.: abx294054

Size: 100 tests

Detection Range: 0.022 mmol/L – 7 mmol/L

Sensitivity: 0.022 mmol/L

Storage: Store the Chromogenic Reagent at 4°C and SA Standard (1 mmol/L) at -20°C. Store both components in the dark.

Application: For detection and quantification of Sialic Acid (SA) concentration in serum, plasma, saliva, tissue homogenates, urine, and hydrothorax samples.

Introduction

Sialic Acid reacts with methyl resorcinol in the presence of an oxidant to form a purple/red colored complex. The absorbance of this complex at 560 nm can be measured and used to calculate the concentration of Sialic Acid according to the Beer-Lambert law of spectrophotometry.

Kit components

1. Chromogenic Reagent: 8 x 60 ml
2. SA Standard (1 mmol/L): 1 ml

Materials required but not provided

1. Spectrophotometer (560 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. High-precision pipette and sterile pipette tips
6. 5 ml EP tubes
7. Centrifuge
8. Vortex mixer
9. Incubator
10. Boiling water bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum**, **Plasma, and Saliva:** Serum, plasma, and saliva samples can be tested directly.
- **Urine:** Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant, keep on ice, and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh at least 30 mg of tissue, and add into normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4) in a ratio of 1 : 9 mass (mg) to volume (µl). For example, per 30 mg of tissue, add into 270 µl of chosen diluent. Homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes, then carefully take the supernatant for analysis. Keep on ice. Assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Other Biological Fluids:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate. Analyse immediately or aliquot and store at -20°C or -80°C.

  Note: To calculate Sialic Acid concentration in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. 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 homogenate	1
Human saliva	1
10 % Mouse liver tissue homogenate	1
10 % Mouse brain tissue homogenate	1
Human hydrothorax	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **SA Standard (1 mmol/L):** Keep on ice during use.
- **Chromogenic Reagent:** Equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

Take care not to collect any precipitate when adding the sample to the cuvette.

Liquid Sample Procedure:

1. Mark microcentrifuge tubes for each standard, sample, and blank.
2. Add 0.1 ml of sample to each sample tube.
3. Add 0.1 ml of SA Standard (1 mmol/L) to each standard tube.
4. Add 0.1 ml of double-distilled water into each blank tube.
5. Add 4 ml of Chromogenic Reagent into each tube. Mix thoroughly and close the top of the tube with plastic film. Create a small hole in the film with a needle.
6. Incubate at 100°C for 15 minutes, then quickly cool with running water.
7. Centrifuge at 2,325 × g for 10 minutes.
8. Set the spectrophotometer to zero with double-distilled water.
9. Measure the OD of each tube with a microplate reader at 560 nm with a 1 cm optical path cuvette.

Tissue Homogenates Procedure:

10. Mark microcentrifuge tubes for each standard, sample, and blank.
11. Add 0.2 ml of sample to each sample tube.
12. Add 0.1 ml of SA Standard (1 mmol/L) and 0.1 ml of double-distilled water to each standard tube.
13. Add 0.2 ml of double-distilled water into each blank tube.
14. Add 4 ml of Chromogenic Reagent into each tube. Mix thoroughly and close the top of each tube with plastic film. Create a small hole in the film with a needle.

15. Incubate at 100°C for 15 minutes, then quickly cool with running water.
16. Centrifuge at 2,325 × g for 10 minutes.
17. Set the spectrophotometer to zero with double-distilled water.
18. Measure the OD of each tube with a microplate reader at 560 nm with a 1 cm optical path cuvette.

C. Calculation of Results

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

$$\text{SA concentration (mmol/L)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}) \times \mathrm{C_1}}{(\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}})}$$

2. Tissue Homogenates:

Sialic Acid activity in tissue samples can be calculated according to total protein concentration, which must be assayed separately (**abx097193**), or according to sample weight.

$$\text{SA concentration (mmol/g protein)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}) \times \mathrm{C_2}}{(\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}) \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Standard}}$	OD value of standard
$\mathrm{OD_{Blank}}$	OD value of blank
$\mathrm{C_1}$	Concentration of standard, 1 mmol/L
$\mathrm{C_2}$	Concentration of standard for tissue samples, 0.5 mmol/L
$\mathrm{C_{Protein}}$	Concentration of protein in sample (g protein/L)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.