Sialic Acid Assay Kit

Catalog No.: abx298966

Size: 96 tests

Detection Range: 0.03 mmol/L – 7 mmol/L

Sensitivity: 0.03 mmol/L

Storage: Store the Standard at -20°C in the dark, and all other components in the dark at 4°C for up to 12 months.

Application: For detection and quantification of Sialic Acid concentration in serum, plasma, tissue, saliva, urine, hydrothorax samples and other biological fluids.

Introduction

Sialic Acid (SA) is a ubiquitous alpha-keto acid found in animal tissues. SA is present in many glycoproteins and oligosaccharides involved in cell communication and recognition. SA plays a role in bacterial evasion of the immune system, 'hiding' mannose antigens that would typically activate complement. Deficiency of SA is implicated in a number of diseases, due to the wide range of functions that SA and its derivatives perform.

Abbexa's Sialic Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Sialic Acid concentration. SA forms a purple-red complex with methyl resorcinol In the presence of an oxidizing agent. The purple-red complex has a maximum absorbance at 560 nm. The concentration of Sialic Acid can be determined by colorimetric measurement of the absorbance at 560 nm. The intensity of the color is proportional to the Sialic Acid concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Detection reagent: 2 x 30 ml
3. Standard (8 mmol/L): 2 x 1 ml
4. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (560 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Water bath / Incubator
7. Centrifuge
8. Vortex mixer
9. Timer

Protocol

A. Preparation of 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Saliva:** Collect plasma using a tube or collection device. Centrifuge for 5 mins at 10,000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Take the supernatant keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 3100 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Hydrothorax samples:** Collect hydrothorax samples into a centrifuge tube with anticoagulant and mix fully. Centrifuge at 10,000 × g for 10 min and discard the supernatant. Take the supernatant keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10,000 × g at 4°C for 15 min. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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
Pig 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% Rat Lung Tissue Homogenate	1
10% Rat Brain Tissue Homogenate	1

Note:

- If dilutions are required, samples should be diluted with Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
- 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 cell lysates and tissue homogenates.

B. Standard Preparation

1. Label 7 vials 1, 2, 3, 4, 5, 6, 7 mmol/L
2. Dilute the 8 mmol/L Standard with double distilled water to the concentrations 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 mmol/L according to the following ratios:

Concentration (mmol/L)	7.0	6.0	5.0	4.0	3.0	2.0	1.0
Standard volume (µl)	175	150	125	100	75	50	25
Double distilled water volume (µl)	25	50	75	100	125	150	175

C. Assay Procedure

Serum, plasma, saliva, and other biological fluids:

1. Set standard, blank and sample tubes. Label the 7 standard tubes 1, 2, 3, 4, 5, 6, 7 mmol/L. Set standard, blank and sample wells on the microplate, and record their positions.
2. Add 25 µl of each standard concentration to the standard tubes.
3. Add 25 µl of each sample solution to the standard tubes.
4. Add 500 µl of Detection Reagent to all tubes.
5. Mix fully with a vortex, and incubate at 100°C in the water bath for 15 minutes. *Ensure that the water level of the water bath is higher than the level of liquid in each tube.*
6. Remove the tubes, and cool with running water. Centrifuge at 2325 × g for 10 minutes. If precipitation is visible, centrifuge again.
7. Take 200 µl of the supernatant of each tube and add to the corresponding microplate wells.
8. Measure the OD of each well with a microplate reader at 560 nm.

Tissue samples:

1. Set standard, blank and sample tubes. Label the 7 standard tubes 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 mmol/L. Set standard, blank and sample wells on the microplate, and record their positions.
2. Add 25 µl of each standard concentration, and 25 µl of double distilled water to the standard tubes.
3. Add 50 µl of each sample solution to the standard tubes.
4. Add 500 µl of Detection Reagent to all tubes.
5. Mix fully with a vortex, and incubate at 100°C in the water bath for 15 minutes. *Ensure that the water level of the water bath is higher than the level of liquid in each tube.*
6. Remove the tubes, and cool with running water. Centrifuge at 2325 × g for 10 minutes. If precipitation is visible, centrifuge again.
7. Take 200 µl of the supernatant of each tube and add to the corresponding microplate wells.
8. Measure the OD of each well with a microplate reader at 560 nm.

D. Calculation of Results

The standard curve should be plotted using the linear equation y = *m*x + *b*. The OD value of the standard should be used as the y axis, and the concentration of the standard should be used as the x axis.

1. Serum, plasma, saliva and other biological fluids:

The standard curve for this sample type is $y = m_1 x + b_1$

$$\mathrm{SA\ (mmol/L)} = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}) - b_1}{m_1} \times f$$

2. Tissues samples:

The standard curve for this sample type is $y = m_2 x + b_2$

$$\mathrm{SA\ (mmol/g)} = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}) - b_2}{m_2 \times C} \times f$$

where:

SA	The concentration of Sialic Acid
$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Blank}}$	OD value of blank
b_1	The intercept of the standard curve, $y = m_1 x + b_1$
m_1	The gradient of the standard curve, $y = m_1 x + b_1$
b_2	The intercept of the tissue standard curve, $y = m_2 x + b_2$
m_2	The gradient of the tissue standard curve, $y = m_2 x + b_2$
C	The concentration of sample (g/L)
f	The dilution factor of sample