Sialic Acid (SA) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Detection instrument: Spectrophotometer/Microplate reader

Cat No: NA0140

Size: 100T/96S

Components:

Extraction Reagent: Liquid 110 mL×1. Storage at 2-8℃.

Chromogenic Solution: Liquid 35 mL×1. Storage at 2-8℃.

Standard: Liquid 0.5 mL×1. Storage at -20℃. 4 mmol/L sialic acid standard solution.

Product Description:

Sialic acid (SA), also known as N-acetylneuraminic acid, is an important component of glycoproteins and glycolipids on the cell membrane. It widely exists in organisms and participates in various physiological functions on the cell surface.

Sialic acid forms a purplish red complex with 5-methylresorcinol in the presence of oxidant. It has a maximum absorption peak at 560nm. The content of sialic acid can be calculated by measuring the light absorption value of the product at 560nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, centrifuge, water bath/constant temperature incubator, transferpettor, mortar/homogenizer/cell ultrasonic crusher, micro quartz cuvette/96 well UV flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation:

1. Tissue

According to the proportion of tissue weight (g): the volume of Extract Solution (mL) is 1:5~10. Suggest add 1 mL of Extract Solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 3000rpm for 10 minutes at 4℃, take the supernatant and put it on ice for testing.

2. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells (10^4): the volume of Extract Solution (mL) is 500-1000:1. Suggest add 1 mL of Extract Solution to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3s, interval 7s, The total time is 3min). Centrifuge at 3000rpm for 10 minutes at 4℃, take the supernatant and put it on ice for testing.

3. Emulsion sample

Centrifuge at 10000g for 10 minutes at 4℃, remove the upper layer of grease and then use it.

4. Other liquid samples

Direct measurement (if there is turbidity, it can be used after centrifugation).

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 560 nm, set zero with distilled water.

2. Add reagents with the following list (reaction in EP tube):

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	10	-	-
Standard	-	10	-
Distilled water	-	-	10
Chromogenic solution	300	300	300

Mix thoroughly, put the reaction solution in 100℃ water bath for 15 minutes(cover tightly to prevent water loss), Centrifuge at 8000rpm for 10 minutes at room temperature (if there is turbidity, the centrifugal speed can be increased). Take the supernatant and detect the absorbance at 560 nm. Record it as A_T, A_S, A_B, $\Delta A=A_T - A_B$, $\Delta A_S=A_S - A_B$ (The standard curve and blank tube only need to be measured 1-2 times).

III. Calculation:

1. Calculate by the Protein concentration (Protein concentration shall be determined by yourself)

Sialic Acid (SA) Content (mmol/g prot) = $\Delta A \times C \div \Delta A_S \times V_S \div (Cpr \times V_S \times 1000)$

$= 0.004 \times \Delta A \div \Delta A_B \div Cpr$

2. Calculate by the Sample weight

Sialic Acid (SA) Content (mmol/g quality) = $\Delta A \times C \div \Delta A_S \times V_S \div W$

$= 0.004 \times \Delta A \div \Delta A_S \div W$

3. Calculate by the number of bacteria or cells

Sialic Acid (SA) Content (mmol/10^4 cell) = $\Delta A \times C \div \Delta A_S \times V_S \div N$

$= 0.004 \times \Delta A \div \Delta A_S \div N$

4. Calculate by the volume of liquid sample

Sialic Acid (SA) Content (mmol/L) = $\Delta A \times C \div \Delta A_S$

$= 4 \times \Delta A \div \Delta A_S$

V_S: Sample volume, 0.001 L;

C: Concentration of standard, 4mmol/L;

Cpr: Sample protein concentration, mg/mL;

W: Weight of the sample, g;

N: Number of cells (Unit: 10^4);

1000: Conversion factor, 1L=1000mL

Notes:

1. The temperature of 100 ℃ boiling water bath is relatively high, so it is recommended to wrap the centrifugal tube with sealing film or use the centrifugal tube with spiral cover.

2. The measured value range of ΔA is 0.01-0.7. If the measured absorbance value exceeds the

absorbance value in the linear range, the sample can be diluted with distilled water and then measured again. If the measured absorbance value is less than the absorbance value in the linear range, the sample size needs to be increased and then measured again. Don't forget to modify the calculation formula.

Experimental instances：

1. Take 10μL bovine serum, operated according to the determination steps. Measured with 96 well UV flat-bottom plate, A_T = 0.227, A_B = 0.046, ΔA = 0.181. A_S = 0.346, $ΔA_S$ = 0.3. Calculate the content according to the serum:

Sialic Acid (SA) Content (mmol/L) = 4×ΔA÷$ΔA_S$ = 2.41 mmol/L.

2. Take 10μL plasma, operated according to the determination steps. Measured with 1mL glass cuvette, A_T = 0.196, A_B = 0.046, ΔA = 0.15. A_S = 0.3465, $ΔA_S$ = 0.3. Calculate the content according to the serum:

Sialic Acid (SA) Content (mmol/L) = 4×ΔA÷$ΔA_S$ = 2.0 mmol/L.

3. Take 0.123 g mouse liver, add 1 mL extract solution to homogenize in ice bath, centrifuge at 4℃ and 3000rpm for 10 min, take the supernatant into another EP tube, operated according to the determination steps. Measured with 1mL glass cuvette, A_T = 0.088, A_B = 0.046, ΔA = 0.042. $ΔA_S$ = 0.3. Calculate the content according to the serum:

Sialic Acid (SA) Content (mmol/L) = 0.004×ΔA÷$ΔA_S$÷W = 0.0046 mmol/L.

References：

[1] Yongpoovorawan, S. O. C. , Role of serum total sialic acid in differentiating cholangiocarcinoma from hepatocellular carcinoma[J]. World Journal of Gastroenterology,2003,9(10):2178-2181.

[2] Lu yiqin , Glycophorin variants and contents of sialic acid and total sulfhydryl groups on erythrocyte membranes of residents in a malaria hyperendemic area[J]. Chinese Medical Journal,1998,111(7):606-609.

Related products：

NA0684/NA0443	β-galactosidase (β-GAL) Activity Assay Kit
NA0209/NA0208	Glycosylated serum protein (GSP) content Assay Kit
NA0852/NA0610	Ascorbate Peroxidase (APX) Activity Assay Kit