ab282912 – Sialic Acid ELISA Kit

For *in vitro* quantitative determination of Sialic Acid in human serum, plasma, tissue homogenates and other biological fluids.
For research use only - not intended for diagnostic use.

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Sialic Acid Micro ELISA Plate	1 unit	4°C
Sialic Acid Lyophilized Standard	2 vials	4°C
Sample / Standard dilution buffer	20 ml	4°C
Biotin-detection antibody (Concentrated)	60 µl	4°C
Antibody dilution buffer	10 ml	4°C
HRP-Streptavidin Conjugate (SABC)	120 µl	4°C
SABC dilution buffer	10 ml	4°C
TMB substrate	10 ml	4°C
Stop Solution	10 ml	4°C
Wash buffer (25X)	30 ml	4°C
Plate sealers	5	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Biotin-detection antibody working solution: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation

1. Centrifuge standard tube for 1min at 10000xg. Label it as Zero tube.
2. Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2min at room temperature. Invert the tube several times to mix gently. (Or mix using a low speed vortex mixer for 3-5 seconds.)
3. Centrifuge the tubes for 1min at 1000xg, so the liquid moves towards the bottom of tube removing possible air bubbles.
4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, and so on until the 1/64 tube. The blank tube should only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 4000ng/ml, 2000ng/ml, 1000ng/ml, 500ng/ml, 250ng/ml, 125ng/ml, 62.5ng/ml, 0ng/ml.

   Δ Note: *Store the zero tube with dissolved standards at 2-8℃ and use it within 12h. Other diluted working solutions containing standards should be used in 2h.*

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1000 x g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: Rinse the tissues with ice-cold PBS (0.01M, pH 7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 x g to retrieve the supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.

Other biological fluids: Centrifuge samples for 20 min at 1000 x g at 4°C. Collect the supernatant and carry out the assay immediately.
Δ Note: *End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

Assay Procedure

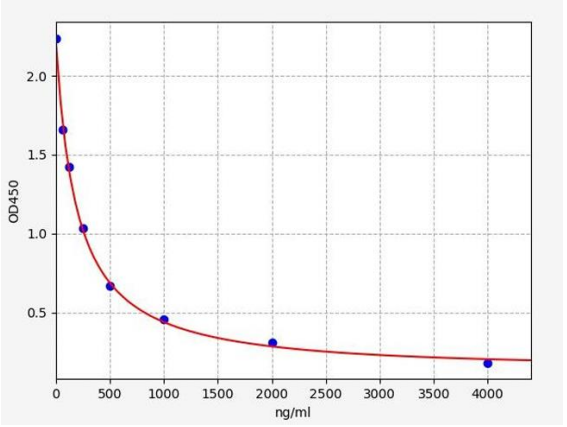
- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. Prepare all reagents, samples, and standards.
2. Wash plate 2 times with 1X Wash Solution before adding standard, sample and control wells.
3. Add 50 µl of each standards or samples into appropriate wells. Immediately add 50 µl of Biotin-detection antibody working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C.
4. Discard the solution and wash 3 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multichannel pipette or auto washer. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
6. Discard the solution and wash 5 times with 1X Wash Solution as step 4.
7. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark within 15-20 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
8. Add 50 µl of Stop Solution to each well. Read result at 450 nm within 20 minutes.

Typical Data & Standard Curve

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average
0	2.206	2.27	2.238
62.5	1.634	1.682	1.658
125	1.403	1.443	1.423
250	1.016	1.046	1.031
500	0.656	0.676	0.666
1000	0.448	0.46	0.454
2000	0.305	0.313	0.309
4000	0.176	0.182	0.179



Δ Note: *If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution.*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Calculations

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis.
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Technical Support

For all technical or commercial enquiries please go to:
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