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Sialic Acid (SA) ELISA Kit

07/16

(Catalog # K4223-100, 100 assays, Store at 4°C)

I. Introduction:

Sialic acids are found widely distributed in animal tissues and to a lesser extent in other organisms, ranging from plants and fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides. In humans the brain has the highest sialic acid concentration, where these acids play an important role in neural transmission and ganglioside structure in synaptogenesis. BioVision's Sialic Acid ELISA kit is a competitive ELISA assay for the quantitative measurement of Sialic Acid in serum, plasma and cell culture supernatants. The density of color is proportional to the amount of Sialic Acid captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Sialic Acid.
Detection Range: 7.813- 500 µg/ml
Sensitivity: < 4.68 µg/ml

III. Sample Type:

Serum, plasma, tissue homogenates and other biological fluids.

IV. Kit Contents:

Components	K4223-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4223-100-1
Lyophilized Standard	2 vials	K4223-100-2
Sample / Standard dilution buffer	20 ml	K4223-100-3
Biotin- detection antibody (Concentrated)	60 µl	K4223-100-4
Antibody dilution buffer	10 ml	K4223-100-5
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µl	K4223-100-6
SABC dilution buffer	10 ml	K4223-100-7
TMB substrate (Avoid light)	10 ml	K4223-100-8
Stop Solution	10 ml	K4223-100-9
Wash buffer (25X)	30 ml	K4223-100-10
Plate sealers	5	K4223-100-11

V. User Supplied Reagents and Equipment:

- 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

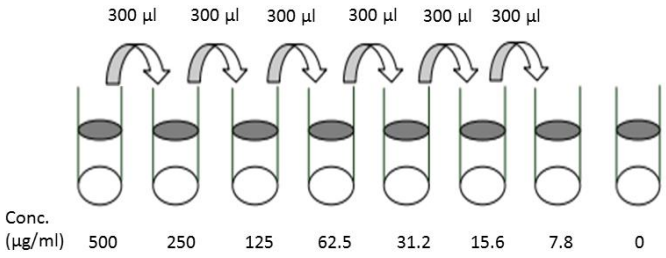
VI. Storage and Handling:

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

VII. Reagent and Sample Preparation:

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

1. **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.
2. **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.
3. **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.
4. **Standard Preparation:**
   - Reconstitute the lyophilized Sialic Acid standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 500 µg/ml standard stock solution. Use within 2 hours after reconstituting.
   - Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.



FOR RESEARCH USE ONLY! Not to be used on humans.

- Prepare 0.6 ml of 250 µg/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 0 µg/ml

5. Sample Preparation:

Note: 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 hour at room temperature or overnight at 4°C. Centrifuge at approximately 1000×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 10000xg. 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×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×g at 4°C. Collect the supernatant and carry out the assay immediately.
- 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.

VIII. Assay Protocol:

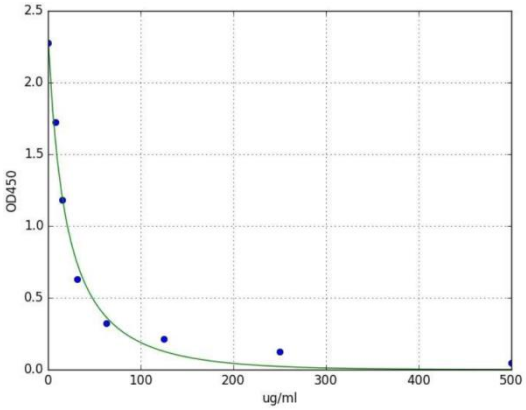
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
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 45minutes 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 multi-channel pipette or autowasher. 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 37ºC 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 ºC 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.

IX. CALCULATION:

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. RELATED PRODUCTS:

- Sialic Acid (NANA) Colorimetric/Fluorometric Assay Kit (Cat. No. K566-100)
- GDNF (Human) ELISA Kit (Cat. No. K4184-100)
- Dopamine (DA) ELISA Kit (Cat. No. K4219-100)