



Neuron Specific Enolase ELISA Kit

(Catalog # E4703-100 ; 96 assays ; Storage at 4°C)

I. Introduction:

BioVision's NSE ELISA kit is a solid phase direct sandwich ELISA. The standards, samples and controls are added into the selected wells which are pre-coated with anti human NSE monoclonal antibody along with an anti-NSE-HRP conjugated pair match antibody. NSE, in the standards, controls and patient samples binds to anti-NSE antibody in the wells and anti-NSE-HRP conjugated antibody binds to the NSE. The unbound glycolytic enzyme enolase, NSE, is washed off by wash buffer. Upon the addition of the TMB substrate, the intensity of color developed is proportional to the concentration of NSE in the samples. A standard curve is prepared relating color intensity to the concentration of the NSE.

II. Sensitivity:

- 2 ng/ml

III. Sample Type:

- Serum

IV. Kit Contents:

Components	E4703-100	Part Number
Microwells coated with Anti-NSE MAb	12 x 8	E4703-100-1
NSE Standard: 6 vials; Frozen	0.25 ml	E4703-100-2
NSE Enzyme Conjugate: 1 bottle (ready to use)	12 ml	E4703-100-3
TMB Substrate: 1 bottle (ready to use)	12 ml	E4703-100-4
Stop Solution: 1 bottle (ready to use)	12 ml	E4703-100-5
20X Wash concentrate: 1 bottle	25 ml	E4703-100-6

V. User Supplied Reagents and Equipment:

- deionized water, plate reader capable of reading absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C

Standards: Standards should remain frozen at or below -70°C until immediately before use. Quickly refreeze unused standard for later use. The Standard set contains human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual,

20X Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

VII. Assay Protocol:

1. Sample preparation: Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 72 hours. If storage time exceeds 72 hours, store frozen at (-20°C or lower) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

2. Standard cure: Check NSE standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached. To construct the standard curve, plot the absorbance for the NSE standards (vertical axis) versus the NSE standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

	Conc. ng/mL	OD 450 nm
Std 1	0	0.010
Std 2	2.5	0.049
Std 3	7.5	0.137
Std 4	17.5	0.362
Std 5	35	0.809
Std 6	75	1.562
Std 7	150	2.167



3. Assay procedure: Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 μ l of NSE standards, control and patient's sera in to selected wells.
3. Add 100 μ l of working solution of anti-NSE enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25 °C), with shaking (600RPM).
5. Remove liquid from all wells. Wash wells three times with 300 μ l of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 μ l of TMB substrate to all wells.
7. Incubate for 30 minutes at room temperature.
8. Add 50 μ l of stop solution to all wells. Shake the plate gently, for 10 seconds, to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

VIII. Related Products:

Human Recombinant Neuron-Specific Enolase (NSE) (6362)

Human Recombinant Alpha-Enolase (6363)

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