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2016-06-08

Cat#: NS193T (96 Tests)
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CALBIOTECH
A life science company

Neuron Specific Enolase (NSE) ELISA

Catalog No.: NS193T (96 Tests)

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with Anti-NSE MAb	12x8x1
2.	NSE Standard: 6 vials; Frozen	0.25ml
3.	NSE Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4.	TMB Substrate: 1 bottle (ready to use)	12ml
5.	Stop Solution: 1 bottle (ready to use)	12ml
6.	20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   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, "Biosafety in Microbiological and Biomedical Laboratories." 1984
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

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).

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 µl of NSE standards, control and patient's sera in to selected wells.
3. Add 100 µl 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 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 30 minutes at room temperature.
8. Add 50 µl 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.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. 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**.**
2. 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.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	Conc. ng/mL	OD 450 nm
Std 1	0	0.008
Std 2	5	0.069
Std 3	15	0.198
Std 4	35	0.500
Std 5	75	1.105
Std 6	150	2.130

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.