DRG® Neuron Specific Enolase (NSE) ELISA (EIA-4424)

USA: RUO

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 NAME AND INTENDED USE

The NEURON-SPECIFIC ENOLASE (NSE) ELISA is a solid phase enzyme linked immunosorbent assay (ELISA).

2 PRINCIPLE OF THE ASSAY

The NSE ELISA is a solid phase enzyme linked immunosorbent assay (ELISA). The samples, standards and controls and biotinylated Anti-NSE antibodies are incubated in the wells. During the incubation, specific NSE bound to anti-NSE antibody on the wells. Unbound NSE antigen is removed by washing the wells with buffer. Enzymes conjugate is added to each well. After the incubation, unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the NSE present in each sample. Upon addition of the substrate and chromogen, the intensity of blue color will develop in proportion to the concentration of NSE antigen in the samples.

3 WARNING AND PRECAUTION

1. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
2. Warning potential bio-hazardous material: The matrix of Negative and Positive controls is human serum. The Standard human serum used found negative HBsAg and HIV and HCV antibodies when tested with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at Bio-safety level 2, as recommended for any potentially infectious human serum of blood specimen in the Center for Disease Control/National Institutes of Health Manual, “Bio-safety in Microbiological and Biomedical Laboratories” 1984.

4 STORAGE AND STABILITY

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The reagents are stable until expiration of the kit.
3. TMB Solution should be colorless; if the Solution turns blue, it must be replaced. Do not expose test reagents to strong light during storage or usage.

5 SAFETY INSTRUCTIONS

1. NSE Standard set is made of human origin and free from HIV, HCV, and HBsAg agents. However, for safety, it must be treated as infectious materials.
2. Do not smoke or eat in areas where specimens or reagent kits are handled.

3. Do not mouth pipette. Wear PVC gloves when handling reagent kits or specimens, and wash hands thoroughly afterwards.
4. Avoid contact of hydrochloric acid with skin and mucous membranes.

6 MATERIALS PROVIDED

1. **Micro-well Strips** (96-wells): Streptavidin coated wells. 96 wells (8 x 12)
2. **Enzyme & Antibody Solution** (11 mL):
   Anti-NSE antibody conjugate with horseradish peroxidase and Biotinylated antibody solution.
3. Reference **Standard** set (0.5 mL/each)
   Calibrated to 5, 25, 50, 100, and 200 µg/L. **Store in freezer in aliquot**.
   The Standards are not stable at room temperature.
4. Low and High **Control** (0.5 mL/each). Value indicates on the vial. **Store in freezer in aliquot.**
   The Controls are not stable at room temperature.
5. **Sample diluent** or zero Standard (11 mL).
6. **TMB Solution** (11 mL): Buffer containing hydrogen peroxide and Tetramethylbenzidine.
7. **Washing buffer** concentrate: (10 mL) (100x).
   Prepare the working washing buffer by adding the 10 mL Washing buffer concentrate into 990 mL distilled water.
8. **Stop Solution**: 2N HCl solution.
9. Well Holder: for securing individual wells.

7 MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader capable of reading at 450nm.
2. Pipettor with tips for 25 µL and 100 µL
3. 1L washing bottle.

8 SAMPLE COLLECTION AND HANDLING

Collect blood aseptically by venipuncture, allow to clot and separate the serum by centrifugation at room temperature. Store in sterile tubes.
Prolonged storage of whole blood can cause release of NSE from blood cells.
If sera cannot be assayed immediately, they can be stored at 2-8°C for one day or frozen at -20°C for up to 30 days prior to assay.
Sample should not be refrozen. Repeated freezing and thawing is not recommended. Do not store in self-defrosting freezer.
Do not use hyperlipemic or hemolyzed samples.
Plasma is not recommended since significant amounts of NSE can be released from the thrombocytes.

9 PREPARATION FOR ASSAY

1. Before beginning the test, bring all samples and reagents to room temperature (24±3°C) and mix each gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent result.
3. Use new disposable tips for each sample.

10 ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense **25 µL** of *references, controls and samples* into the appropriate wells.
3. Immediately dispense **100 µL** of *Enzyme and AB Solution* into each well.
4. Incubate for 60 minutes at room temperature.
5. Remove incubation mixture and rinse the wells 5 times with washing buffer. (300 µL/well/each rinse).
6. Dispense **100 µL** of **TMB Solution** into each well.
7. Incubate for 30 minutes at room temperature.
8. Stop reaction by adding **50 µL** of *Stop Solution* each well and read absorbance at 450 nm with microplate reader.

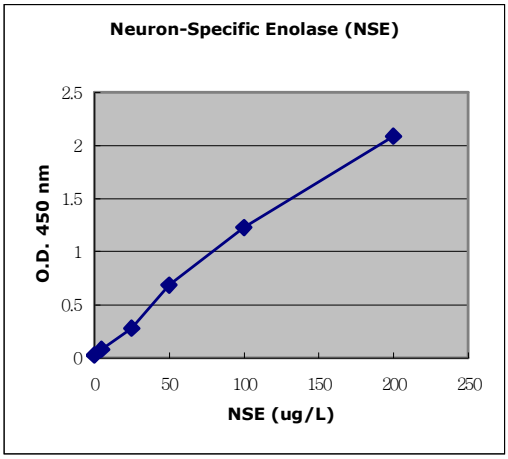
11 PROCEDURE NOTE

1. Wash the microwells and remove water thoroughly.
2. Pipet all reagents and samples into bottom of well. Vortex-mixing or shaking is not required.
3. Absorbance is the function of the time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready to ensure the equal elapsed time for each pipeting without interruption.
4. For the same reason, run no more than 20 samples with a set of reference standards induplicate for each assay.
5. If a serum specimen contains greater that 200 µg/L of NSE, the sample must be diluted with sample diluent and re-assayed as described in the assay procedure.

12 CALCULATION OF RESULTS

1. Plot the concentration (X) of each standard against its absorbances (Y) on graph paper.
2. Obtain the NSE value of sample by reference to the standard curve as follows (these data are for demonstration purposes only. It must not be used in place of date generated for each assay).

Well #	Description (µg/L)	Absorbance (450 nm)	NSE Value (µg/L)
B1	0	0.021	
C1	5	0.080	
D1	25	0.277	
E1	50	0.683	
F1	100	1.226	
G1	200	2.084	
H1	Sample A	0.244	18.3
A3	Sample B	1.087	94.2



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