DRG

DRG® NSE ELISA (EIA-4610)

CE

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RUO in the USA

This kit is intended for Research Use Only.

Not intended for use in diagnostic procedures.

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

1 INTENDED USE

Measurement of hNSE concentration in human serum. NSE ELISA kit is intended for laboratory use only.

2 PRINCIPLE

The NSE ELISA test is based on simultaneous binding of human Neuron Specific Enolase by two monoclonal antibodies, one immobilized on microwell plates and the other conjugates with horseradish peroxidase (HPR).

After incubation the bound/free separation is performed by a simple solid-phase washing, then the TMB-Substrate solution (TMB) is added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbancies are determinated.

The colour intensity is proportional to the hNSE concentration in the sample.

hNSE concentration in the sample is calculated based on a Calibration curve.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **Standards (CAL 0 - CAL4)**, 2 vials each Calibrator,
   lyophilized;
   please read carefully paragraph 6.1
2. **Controls**, 2 vials each, lyophilized
   Negative and Positive Control
   please read carefully paragraph 6.1
3. **Incubation Buffer**, 1 vial, 50 mL,
   Phosphate buffer 50 mM, pH 7.4, BSA (1 g/L)
4. **Conjugate**, 1 vial, 1 mL,
   Monoclonal anti hNSE antibody conjugated with horseradish peroxidase (HRP)
5. **Microplate**, 1 breakable microplate,
   Monoclonal anti hNSE antibody adsorbed on the microplate
6. **TMB-Substrate**, 1 vial, 15 mL,
   H_2O_2-TMB 0.26g/L, (avoid any skin contact)
7. **Stop Solution**, 1 vial, 15 mL,
   Sulphuric acid 0.15 mol/L, (avoid any skin contact)

8. **Wash Solution 50X** concentrate, 1 vial, 20 mL
   NaCl (45 g/L); Tween 20 (55 g/L)

3.2 Necessary reagents not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.

Microplate reader (450 nm, 620-630 nm)

Note

The Calibrators and Controls contain hNSE in a proteic stabilizing matrix solution.

Store all reagents between 2 °C - 8 °C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, the plate is stable up to expiry date.

4 WARNINGS

- Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300 as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- **Calibrator values are lot-specific**.

5 PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2 °C - 8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22 °C - 28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.

– The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
– It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
– Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
– Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
– Maximum precision is required for reconstitution and dispensation of reagents.
– Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
– Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 Preparation of Standards and Controls

Reconstitute Standards and Controls with 0.75 mL of deionized H_2O before use.

Important note: Reconstituted Calibrators and Controls are very sensitive to temperature, so you should proceed as follows:

1. Reconstitute Standards and Controls with 0.75 mL of deionized water
2. Leave on a rolling mixer for about 5 minutes
3. Take the necessary aliquot for the assay and **immediately** aliquot and freeze at -20 °C unused Standards and Controls.

Reconstituted Standards and Controls are stable 1 month at -20 °C; avoid repeated freezing and thawing.

The Calibrators have **approximately** the following concentrations:

	CAL 0	CAL 1	CAL 2	CAL 3	CAL 4
ng/mL	0	4	20	50	100

The right concentrations for the curve compute are lot specific and are printed on the Standard vial labels.

6.2 Diluted Conjugate

Prepare immediately before use.

Add 20 µL of Conjugate (reagent 4) to 1 mL of Incubation Buffer (reagent 3), the quantity to prepare is directly proportional to the number of test.

Mix gently leaving in a rotating shaker for at least 5 minutes.

6.3 Preparation of Wash Solution

Dilute contents of wash buffer concentrate (50X) to 1000 mL with distilled or deionised water in a suitable storage container.

For smaller volumes respect the dilution ratio of 1:50.

The diluted buffer is stable at 2 °C - 8 °C for at least 30 days.

6.4 Preparation of the Sample

The hNSE determination can be carried out in human serum.

The serum would have to be separated from the blood within 60 minutes in order to avoid the increment of the hNSE from the blood cells release.

Do not use hemolyzed samples.

Avoid use of plasma since meaningful amounts of hNSE could be yielded from platelets.

Samples can be stored at 2 °C - 8 °C for 1 day; for long periods store at -20 °C.

Avoid repeated freeze-thaw cycles. Do not allow the samples at room temperature for long period.

6.5 Procedure

Allow all reagents to reach room temperature (22 °C - 28 °C).

At the end of the assay, store immediately the reagents at 2 °C - 8 °C avoid long exposure to room temperature (see paragraph 6.1 for Calibrators and Controls).

Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2 °C - 8 °C.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (CAL 0 - CAL 4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
CAL 0 - CAL 4	**25 µL**		
Sample/ Controls		**25 µL**	
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate at room temperature (22 °C - 28 °C) for 1 hour. Remove the contents from each well and wash the wells 3 times with 300 µL of diluted Wash Solution. Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**

Incubate at room temperature (22 °C - 28 °C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7 QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of hNSE for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8 RESULTS

8.1 Mean Absorbance

Calculate the mean of the absorbancies (Em) corresponding to the single points of the calibration curve (CAL 0 - CAL 4) and of each sample.

8.2 Calibration curve

Plot the values of absorbance (Em) of the Calibrators (CAL 0 - CAL 4) against concentration. Draw the best-fit curve through the plotted points. (e.g.: Cubic Spline, Sigmoid Logistic or Four Parameter Logistic).

8.3 Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

Each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

9 WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

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