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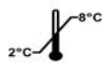
Instructions for use

h-NSE ELISA

REF TM E-4700R



RUO

For research use only – Not for use in diagnostic procedures

NSE ELISA

1. INTENDED USE

Immunoenzymatic colorimetric method for determination 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 Standard curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

Standards and **Controls** - lyophilized*

Cat. no.	Symbol	Standard	concentration **	Volume / Vial
TM E-4701	STANDARD A	Standard A	0 ng/ml	2 x 0.75 ml
TM E-4702	STANDARD B	Standard B	4 ng/ml	2 x 0.75 ml
TM E-4703	STANDARD C	Standard C	20 ng/ml	2 x0.75 ml
TM E-4704	STANDARD D	Standard D	50 ng/ml	2 x 0.75 ml
TM E-4705	STANDARD E	Standard E	100 ng/ml	2 x 0.75 ml
TM E-4751	CONTROL 1	Control 1	*The right concentration are stated on the standard vial label and on the QC-Report*	2 x 0.75 ml
TM E-4752	CONTROL 2	Control 2		2 x 0.75 ml

** please read carefully paragraph 6.1*

***approximately concentration: the right concentration for the curve compute are lot-specific and are stated on the standard vial labels and on the QC-Report.*

TM E-4713 INC-BUFF **Incubation Buffer**
Content: Phosphate buffer (50 mM), pH 7.4; BSA (1 g/l)
Volume: 1 x 50 ml

TM E-4740 CONJUGATE-CONC **Conjugate**
Content: Monoclonal anti hNSE antibody conjugated with horseradish peroxidase (HRP)
Volume: 1 x 1 ml

TM E-4731 96 **Microplate**
Content: 1 breakable microplate, Monoclonal anti hNSE antibody adsorbed on the microplate

MS E-0055 SUBSTRATE **TMB Substrate**
Content: H_2O_2-TMB 0.26g/l (avoid any skin contact)
Volume: 1 x 15 ml

MS E-0080 STOP-SOLN **Stop Solution**
Content: Sulphuric acid 0.15 mol/l, (avoid any skin contact)
Volume: 1 x 15 ml
Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

SA E-0030 WASH-CONC 50x **Wash Solution** - 50x concentrated

Content: NaCl (45 g/l); Tween 20 (55 g/l)

Volume: 1 x 20 ml

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 Standards 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

- This kit is intended for research use only.
- 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.
- This method allows the determination of hNSE inside the range of Standard A – Standard E. **Standards 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. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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 lipaemic 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 each vial of Standard and Control with 0.75 ml of deionized H_2O before use.

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

1. Reconstitute each vial of Standard and Control 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 Standards have **approximately** the following concentrations:

	Standard A	Standard B	Standard C	Standard D	Standard E
ng/ml	0	4	20	50	100

The right Standard concentrations for the curve compute are lot specific and are stated on the Standard vial labels and on the QC-Report.

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) for at least 30 minutes.
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 Standards 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 standard curve (Standard A – Standard E), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample / Controls	Blank
Standard A – Standard E	**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. Automatic washer: if you use automated equipment, wash the wells at least 5 times.			
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 standard 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 standard curve (Standard A – Standard E) and of each sample.

8.2 Standard curve

Plot the values of absorbance (Em) of the Standards (Standard A – Standard E) 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 standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

9. REFERENCE VALUES

The serum values are comprised in the following intervals:

	hNSE
Normal range	0 - 12 ng/ml
Pathological value	> 12 ng/ml

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation.
Therefore, 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.

10. PERFORMANCE AND CHARACTERISTICS

10.1 Specificity

The antibody is directed specifically against the human neuron specific enolase.
Cross reactivity values have been calculated on a weight/weight basis.

NSE Fitzgerald (Cat.No.30AN10 Lot. A99052602)	100 %
NNE Biogenesis (Cat. 6880-1004 Lot. 991105A)	<0.22 %

10.2 Sensitivity

The lowest detectable concentration of hNSE that can be distinguished from the Standard A is 0.19 ng/ml at the 95 % confidence limit.

10.3 Precision

10.3.1 Intra-assay

Within run variation was determined by replicate measurements (16x) of two different control sera in one assay.
The within assay variability is ≤ 4.4%.

10.3.2 Inter-assay

Between run variation was determined by replicate measurements (10x) of two different control sera in different lots. The between assay variability is ≤ 11.2%.

10.4 Correlation

The NSE ELISA (x) kit was compared to another commercially available hNSE assay (y). 28 serum samples were analysed according in both test systems.
The linear regression curve is:
$(y) = 1.34 \times (x) - 0.66$
$r^2 = 0.971$

10.5 Hook Effect

This NSE ELISA kit shows no Hook Effect up to 5000 ng/ml of hNSE.

11. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

12. LITERATURE

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7. Ramont L, Thoannes H, Volondat A, Chastang F, Millet MC, Maquart FX. Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice. Clin Chem Lab Med. 2005;43(11):1215-7.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!