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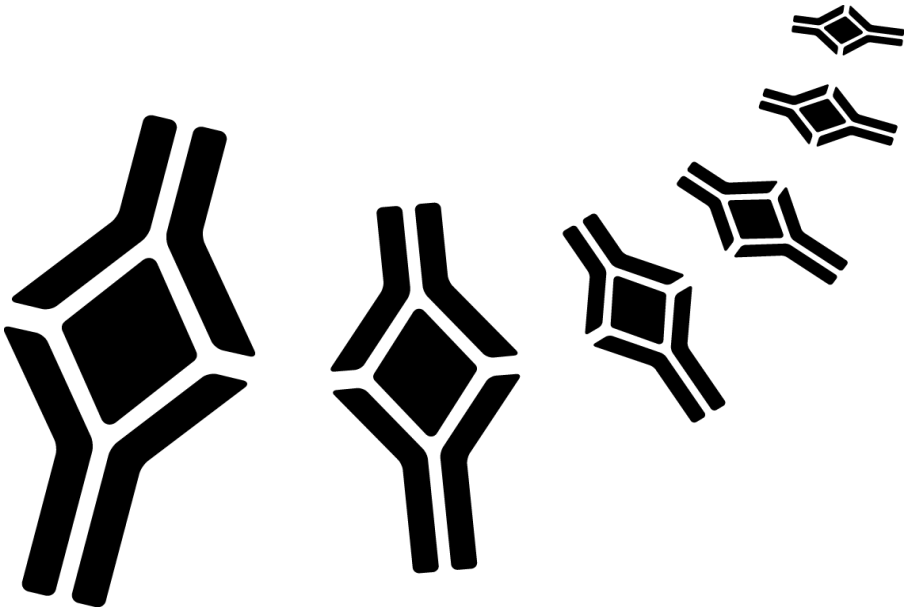

HUMAN NSE ELISA

Product Data Sheet

Cat. No.: RIM002R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of human NSE ELISA concentration in human serum.
Human NSE ELISA kit is intended for research use only.

2. INTRODUCTION

Neuron Specific Enolase (2-phospho-D-glycerate hydrolase) is an isoenzyme that belongs to the enolase family (homo- and heterodimer constituted of α, β and γ subunit) that is distingued from these by the presence of the specific γγ heterodimer.
The clinical usefullness of human NSE like tumor marker is compared to non small cell lunge cancer (NSCLC), to neuroblastoma, to medullary carcinoma of the thyroid, pancreatic islet cell tumor and to non neoplastic condition of neuronal disease and cerebral trauma.
The human NSE ELISA test cannot be used as a screening test for neuroendocrine tumors, but may be used to follow levels in established diagnosis.

3. TEST PRINCIPLE

The human 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 human NSE concentration in the sample.
The human NSE concentration in the sample is calculated based on a series of Calibrator curve.

4. 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- 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 (22-28°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 lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

5. REAGENT SUPPLIED

5.1 Reagents and materials supplied in the kit

1. **Human NSE Calibrators** (2 vials of each calibrator, lyophilized). Please read carefully chapter 7.)
   CAL_0
   CAL_1
   CAL_2
   CAL_3
   CAL_4

2. **Controls** (2 vials for each control, lyohpilized)
   Negative Control
   Positive Control

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 human NSE antibody conjugated with horseradish peroxidase (HRP)

5. **Coated Microplate** (1 breakable microplate)
   Monoclonal anti human NSE antibody adsorbed on microplate

6. **TMB Substrate** (1 vial, 15 mL)
   H_2O_2-TMB (0,26 g/L) *(avoid any skin contact)*

7. **Stop Solution** (1 vial, 15 mL)
   Sulphuric acid 0,15M *(avoid any skin contact)*

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

5.2 Necessary reagnets not supplied

Distilled water.

5.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplate reader (450 nm, 60-630 nm).

Note

The calibrators and Controls contain human NSE in a proteic stabilizing matrix solution.
Store all reagents at 2÷8°C in the dark. Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, the microplate is stable until the expiry date of the kit. Do not remove the adhesive sheets on the unutilised strips.

6. WARNINGS

- This kit is intended for research use professional persons only. 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 300R as preservative. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- 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/H2O2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Human NSE inside the range Calibrator 0-4. The calibrators value is lot-specific.

7. PREPARATION OF REAGENTS

- **Preparation of Calibrators (C_0…C_4) and Controls**

Reconstitute Calibrators and controls with 0.75 mL of deionized H2O before use.
Important note: reconstituted Calibrators, and Controls are very sensitive to temperature, so you should precced as follows:

1. Reconsitute Calibrators and Controls wiht 0.75 ml of deionized water
2. Leave on a rolling mixer for about 5 minutes
3. Také the necessary aliquot for the assay and **immediately** aliquot and freeze at - 20°C unused Calibrators and Controls.

Reconsituted Calibrators and Controls are stable 1 month at - 20°C; avoid repeated Fredin and thawing.
The Calibrators have approximately the following concentrations:

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	4	20	50	100

The right concentrations for the curve compute are lot specific and are printed on the Calibrators vial labels and Quality control Sheet.

- **Diluted conjugate**

Prepare immediately before use.
Add 20 µL of Conjugate (reagent 3) to 1 mL of Incubation Buffer (reagent 2), the quantity to prepare is directly proportional to the number of test.
Mix gently leaving in a rotating shaker for at least 5 minutes.

- **Preparation of the 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 2-8°C for at least 30 days.

- **Sample Preparation**

The human NSE 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 human NSE from the blood cells release.
Do not use hemolyzed samples. Avoid use of plasma since meaningful amounts of human NSE could be yielded from platelets.
Samples can be stored at 2÷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.

- **Procedure**
  1. **Allow all reagents to reach room temperature (22-28°C) for least 30 minutes.** At the end of the assai, store immediately the reagents at 2-8°C; avoid long exposure to room temperature (see Preparation of Calibrators and Controls)
  2. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
  3. To avoid potential microbial and/or chemici contamination, unused reagents should never be transferred into the original vials.
  4. 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 (C0-C4), two for each Control, two for each sample, one for Blank.

Reagents	**Calibrator**	**Sample/ Controls**	**Blank**
Calibrator C_0-C_4	25 µL		
Sample /Controls		25 µL	
Diluted Conjugate	100 µL	100 µL	
Incubate at room temperature (22-28°C) for 1 hour. Remove the content from each well 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 for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelenght of 620-630 nm or against Blank within 5 minutes.			

8. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of human NSE for monitoring assai 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 assai 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.

9. CALCULATIONS

9.1 Mean Absorbance

Calculate the mean of the absorbancies (Em) corresponding to the single points to the calibration curve (C0-C4) and of each sample..

9.2 Calibration Curve

Plot the values of absorbance (Em) of the Calibrators (C0-C4) against concentration. Draw the best-fit curve through the plotted points. (es: Cubic Spline, Sigmoid Logistic or Four Parameter Logistic).

9.3 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.

10. REFERENCE VALUES

Normal range	0-12 ng/mL
Pathological value	> 12 ng/mL

Please pay attention to the fact that the determinativ 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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

11. PERFORMANCE CHARACTERISTICS

- **Precision**

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%.

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%.

- **Sensitivity**

The lowest detectable concentration of human NSE that can be distinguished from the Calibrator 0 is 0.19 ng/mL at the 95 % confidence limit.

- **Specificity**

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

- **Correlation**

The human NSE ELISA (BioVendor) kit was compared to another commercially available human NSE assay. 28 serum samples were analysed according in both test systems.
The linear regression curve is: (Commercial kit) = 1.34*(our kit) – 0.66 r2 = 0.971

- **Hook Effect**

human NSE kit shows no Hook Effect up to 5000 ng/mL of human NSE.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

13. TROUBLESHOOTING

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers
- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

14. REFERENCES

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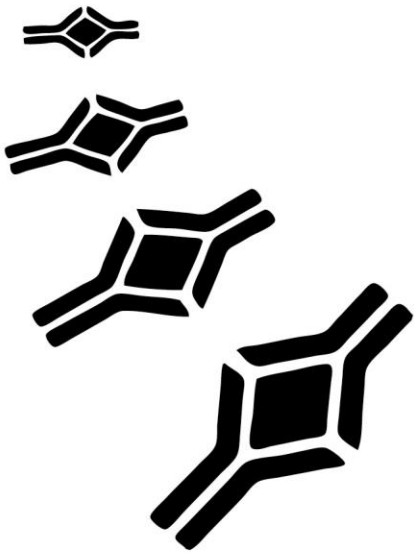

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