ENO2 (Human) ELISA Kit

Catalog Number KA1910

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

Immunoenzymatic colorimetric method for quantitative determination of hNSE concentration in human serum.
ENO2 (Human) ELISA Kit is intended for laboratory use only.

Background

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 usefulness of hNSE 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 NSE ELISA test cannot be used as a screening test for neuroendocrine tumors.

Principle of the Assay

The ENO2 (Human) ELISA Kit 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.

General Information

Materials Supplied

List of component

Component	Amount
Standard A (Standard 0) (Lyophilized): Reconstitute with 0.75 mL/vial deionized water.	2 vials
Standard B (Standard 1) (Lyophilized): Reconstitute with 0.75 mL/vial deionized water.	2 vials
Standard C (Standard 2) (Lyophilized): Reconstitute with 0.75 mL/vial deionized water.	2 vials
Standard D (Standard 3) (Lyophilized): Reconstitute with 0.75 mL/vial deionized water.	2 vials
Standard E (Standard 4) (Lyophilized): Reconstitute with 0.75 mL/vial deionized water.	2 vials
Control 1 (Lyophilized): Negative control. Reconstitute with 0.75 mL/vial deionized water.	2 vials
Control 2 (Lyophilized): Positive control. Reconstitute with 0.75 mL/vial deionized water.	2 vials
Incubation buffer: Phosphate buffer (50 mM), pH 7.4; BSA (1 g/L)	50 mL
Conjugate concentrate: Monoclonal anti hNSE antibody conjugated with horseradish peroxidase (HRP).	1 mL
Microplate: Breakable microplate, Monoclonal anti hNSE antibody adsorbed on the microplate.	96 (8x12) wells
Substrate Solution: H_2O_2-TMB (0.26 g/L) (avoid any skin contact).	15 mL
Stop Solution: Sulphuric acid (0.15 mol/L) (avoid any skin contact).	15 mL
Wash Solution: 50x concentrate, NaCl (45 g/L); Tween 20 (55 g/L)	20 mL

Note: The Standards and Controls contain hNSE in a proteic stabilizing matrix solution.

Storage Instruction

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

Open the bag of 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.

Materials Required but Not Supplied

- ✓ Distilled water
- ✓ Microplate reader (450 nm, 620-630 nm)
- ✓ Automatic dispenser

Precautions for Use

1. This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.

2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. Some reagents contain small amounts of Proclin 300 as preservative. Avoid the contact with skin or mucosa.
5. 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.
6. 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.
7. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
8. This method allows the determination of hNSE inside the range of Standard A – Standard E. Standard values are lot-specific.
9. 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.
10. 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.
11. Allow all kit components and specimens to reach room temperature (22 °C - 28 °C) and mix well prior to use.
12. 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.
13. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
14. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
15. 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
16. 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.
17. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
18. Maximum precision is required for reconstitution and dispensation of reagents.
19. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
20. Plate readers measure vertically. Do not touch the bottom of the wells.

Assay Protocol

Reagent Preparation

✓ Preparation of Standards and Controls

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

Reconstitute Standards and Controls with 0.75 mL of deionized water

Leave on a rolling mixer for about 5 minutes.

Take the necessary aliquot for 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 concentrations for the curve compute are lot specific and are printed on the standard vial labels.

✓ Diluted Conjugate

Prepare immediately before use.

Add 20 µL of Conjugate concentrate to 1 mL of Incubation Buffer, 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 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 hNSE determination must be carried out on 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 2-8°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.

Assay Procedure

- ✓ Allow all reagents to reach room temperature (22-28°C).
- ✓ At the end of the assay, store immediately the reagent at 2-8°C avoid long exposure to room temperature
- ✓ Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°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 Conjugated	100 µL	100 µL	
Incubate at room temperature (22-28°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 (22-28°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 5 minutes.			

- ✓ 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.

Data Analysis

Calculation of Results

- ✓ Mean Absorbance
  Calculate the mean of the absorbancies (Em) corresponding to the single points to the standard curve (Standard A-Standard E) and of each sample.

- ✓ 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. (es: Cubic Spline, Sigmoid Logistic or Four Parameter Logistic).

- ✓ 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.

- ✓ References Values
  The serum values are comprised in the following intervals:
  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.

Performance Characteristics

- ✓ Specificity
  The antibody is directed specifically against the human neuron specific enolase.

- ✓ 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.

✓ 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%.

✓ Correlation

This kit was compared to another commercially available hNSE assay. 28 serum samples were analysed according in both test systems.

The linear regression curve is:

Commercial available hNSE assay = 1.34 x (This kit) - 0.66

$r^2 = 0.971$

✓ Hook Effect

This ENO2 (Human) ELISA Kit shows no Hook Effect up to 5000 ng/mL of hNSE.

✓ Waste Management

Reagents must be disposed of in accordance with local regulations.

Resources

Plate Layout

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11								
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	A	B	C	D	E	F	G	H