NEURON SPECIFIC ENOLASE ELISA

For the quantitative determination of human neuron specific enolase levels in serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 43-NSEHU-E01

Size: 96 wells

Version: V6/US/2019-03- ALPCO April 5, 2019

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human neuron specific enolase (NSE) levels in serum samples.

SUMMARY OF PHYSIOLOGY

The glycolytic enzyme enolase (2-phospho-D-glycerate hydro-lyase) exists as several dimeric isoenzymes (αα, αβ, ββ and γγ) composed of three distinct subunits: α, β, and γ. Three isoenzymes are found in human brain: αα, αβ, and γγ. The heterologous αγ-isoenzyme and the homologous γγ-enolase isoenzymes are known as neuron-specific enolase (NSE) as these isoenzymes initially were detected in neurons and neuroendocrine cells. This assay detects both the αγ and the γγ forms by using monoclonal antibodies specific to the γ-subunit of the enzyme.

Research demonstrates that NSE may be a valuable tumor biomarker of neuroendocrine origin, particularly in small cell lung cancer and in neuroblastoma.

ASSAY PRINCIPLE

This ELISA is designed, developed, and produced for the quantitative measurement of human NSE in serum samples. The assay utilizes the two-site “sandwich” technique with two selected monoclonal antibodies that bind to different epitopes of the γ-subunit of the enzyme.

Assay standards, controls, and samples are added directly to microplate wells that are coated with streptavidin. Subsequently, a mixture of a biotinylated NSE specific monoclonal antibody and a horseradish peroxidase (HRP) labeled NSE specific monoclonal antibody is added to each microplate well. After the first incubation a “sandwich” immunocomplex of “streptavidin-biotin-monoclonal antibody-human NSE-monoclonal antibody-HRP” is formed. The unbound monoclonal antibodies are removed in the subsequent washing step. For the detection of this immunocomplex, the wells are then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the NSE on the wall of the microplate well is directly proportional to the amount of NSE in the sample. A standard curve is generated by plotting the absorbance versus the respective human NSE concentration for each standard on point-to-point, cubical scales, or 4-parameter curve fit. The concentration of human NSE in test samples is determined directly from this standard curve.

REAGENTS:

Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit, refer to the label on the kit box. All components are stable until this expiration date.

Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

Reagents Provided

1. Streptavidin Coated Microplate (Cat. No. 10040)

One microplate with 12 x 8 strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. NSE Tracer Antibody (Cat. No. 30239)
One vial containing 0.6 mL HRP labeled anti-human NSE specific monoclonal antibody in a stabilized protein matrix. This reagent must be diluted with NSE Capture Antibody before use. The dilution procedure is in the assay procedure section. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. NSE Capture Antibody (Cat. No. 30238)
One vial containing 12 mL of biotinylated NSE capture antibody. It should be only used after mixing with NSE tracer antibody. The reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. Wash Concentrate (Cat. No. 10010)
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

5. HRP Substrate (Cat. No. 10020)
One bottle contains 12 mL of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. Stop Solution (Cat. No. 10030)
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. NSE Standards (Cat. No. 30231 – 30235)
Two sets of five vials each containing human NSE in a lyophilized bovine serum-based matrix with a non-azide, non-mercury based preservative. **Refer to vial for exact concentration for each standard.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

8. NSE Controls (Cat. No. 30236 – 30237)
Two sets of two vials each containing human NSE in a lyophilized bovine serum based matrix with a non-azide, non-mercury based preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for research use only. Source material of bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation upon contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL,1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microplate plate cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SAMPLE COLLECTION

Only 20 µL of human serum is required for human NSE measurement in duplicate. No special preparation of the individual is necessary prior to sample collection. Sample should not be taken from donors taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to sample collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum of 30 minutes at room temperature before the serum is separated by centrifugation (850-1500 xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 - 8ºC if the assay is to be performed within 24 hours. Otherwise, samples should be stored at -20ºC or below until measurement. Avoid any repeated freezing and thawing of the sample.

Plasma samples are not recommended for NSE measurement.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
2. Wash Concentrate must be diluted to working strength solution prior to use. Please see REAGENTS section for details.
3. Reconstitute all assay standards and controls by adding **0.5 mL** of deminerialized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solids are dissolved completely prior to use. **These reconstituted standards and controls should be stored at 2-8°C for up to 30 days**. It is not recommended to freeze the reconstituted standards and controls.

2. Assay Procedure

1. Place a sufficient number of streptavidin coated microwell strips in a holder to run human NSE standards, controls, and unknown samples in duplicate.

2. Plate Map

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 4
G	STD 4	SAMPLE 1	SAMPLE 5
H	STD 4	SAMPLE 1	SAMPLE 5

3. Prepare NSE Tracer Antibody and Capture Antibody working solution by 1:21 fold dilution of the Tracer Antibody (30239) with the biotinylated Capture Antibody (30238). For each strip, mix 1 mL of the Capture Antibody with 50 µL of the Tracer Antibody in a clean test tube.
4. Add **10 µL** of standards, controls, and samples into the designated microwells.
5. Add **100 µL** of above mixture of Tracer Antibody and Capture Antibody solution to each of the wells.
6. Cover the plate with the plate sealer and incubate plate at room temperature, shaking at 170 rpm for **1 hour**.
7. Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of HRP Substrate into each of the wells.
9. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
10. Incubate plate at room temperature for **10 minutes or less.**
11. Remove the aluminum foil and plate sealer. Add **100 µL** of Stop Solution into each of the wells. Mix gently.
12. Read the absorbance at 450 nm within 10 minutes in a microplate reader.
13. *NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.*

PROCEDURAL NOTES

1. It is recommended that all standards, controls, and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused streptavidin coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

CALCULATION OF RESULTS

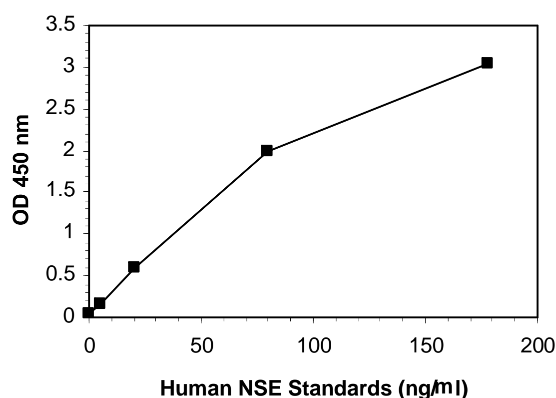
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain the corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using **Point-to-Point** curve fit.

The human NSE concentrations for the controls and samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from human NSE ELISA are represented. **This curve should not be used in lieu of a standard curve run with each assay.**

Well ID	OD 450 nm Absorbance			Results
	Readings	Average	Corrected	
0 ng/mL	0.042 0.043	0.043	0	
5 ng/mL	0.170 0.162	0.166	0.123	
20 ng/mL	0.597 0.857	0.592	0.549	
80 ng/mL	2.058 1.899	1.979	1.936	
178 ng/mL	3.040 3.040	3.04	2.997	
control 1	0.359 0.358	0.359	0.316	11.27 ng/mL
control 2	2.803 2.914	2.859	2.816	147.24 ng/mL



LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human NSE measurement, the values of assay standards were established by correlation to a highly purified NSE standard.
2. For sample values reading greater than the highest standard, it is recommended to dilute the samples and reanalyze them.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents, may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known NSE levels. We recommend that all assays include the laboratory's own or commercial NSE controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the human NSE ELISA as determined by the 95% confidence limit on 20 duplicate determinations of zero standard is approximately 1.2 ng/mL.

High Dose "Hook" Effect

This assay has showed that it does not have any high dose "hook" effect up to 20,000 ng/mL.

Precision

The intra-assay precision is validated by measuring two control samples in a single assay with 20 replicate determinations.

Mean NSE Value (ng/mL)	CV (%)
11.24	4.0
132.16	3.5

The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays.

Mean NSE Value (ng/mL)	CV (%)
11.37	5.99
144.98	4.85

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

#	Dilution	Observed Value	Expected Value	Recovery %
1	Neat	178	-	-
	1:2	85.47	89	96
	1:4	45.38	44.5	102
	1:8	22.4	22.25	101
2	Neat	146	-	-
	1:2	71.44	73	98
	1:4	34.62	36.5	95

Recovery

Two serum samples were spiked with various amounts of human NSE (1 vol. + 1 vol. mixture) and assayed. The results in the value of ng/mL are as follows:

#	Sample	Spiked Sample	Observed Value	Expected Value	Recovery %
1	Sample 1	Sample 3	10.32	10.14	102
		Sample 4	20.78	21.68	96
2	Sample 2	Sample 3	10.07	10.23	98
		Sample 4	23.88	20.35	117

REFERENCES

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Short Assay Procedure:

1. Add 10 µL of standards, controls, and serum samples into the designated microwell.
2. Add 100 µL of the tracer/capture antibody mixture to each well.
3. Cover with a plate sealer and incubate the plate at room temperature, shaking at 170 rpm for 1 hour.
4. Wash each well 5 times.
5. Add 100 µL of HRP Substrate to each of the wells.
6. Cover with a plate sealer and aluminum foil and incubate plate at room temperature for 10 minutes or less.
7. Add 100 µL of Stop Solution to each of the wells.
8. Read the absorbance at 450 nm within 10 minutes.