

EDI™ Human Neuron Specific Enolase ELISA Kit Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Neuron Specific Enolase (NSE) Level in Serum



KT 817

EU:



INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human neuron specific enolase (NSE) levels in patient serum samples. The test might be used as an aid for detecting patients with neuroendocrine differentiated tumors such as small cell lung cancer and neuroblastoma, melanoma, seminoma; and with injury of central nervous system such as traumatic brain injury (TBI).

SUMMARY OF PHYSIOLOGY

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolyase) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\beta\beta$ and $\gamma\gamma$) composed of three distinct subunits: α , β , and γ . Three isoenzymes are found in human brain: $\alpha\alpha$, $\alpha\beta$, and $\gamma\gamma$. The heterologous $\alpha\gamma$ -isoenzyme and the homologous $\gamma\gamma$ -enolase isoenzymes are known as neuron-specific enolase (NSE) as these isoenzymes initially were detected in neurons and neuroendocrine cells. This test detects both the $\alpha\gamma$ and the $\gamma\gamma$ forms by using monoclonal antibodies specific to the γ -subunit of the enzyme.

NSE levels are quite low in normal healthy people and in people with benign disease. Lung cancer is one of the most common cancer forms with incidences about 50-100 per 100,000 population. Approximately 20% of the lung cancer is small cell lung cancer. NSE has been shown to be a valuable tumor marker of neuroendocrine origin, particularly in small cell lung cancer and in neuroblastoma. Although NSE is similar to Chromogranin A in detecting small cell lung cancer and neuroblastoma, Chromogranin A seems better in detecting carcinoid.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human NSE in serum sample. 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 patient samples are added directly to wells of microplate that is coated with a 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 microtiter 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 well is 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 microtiter 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.

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

One microplate with 12 x eight 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 section of assay procedure. 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. ELISA 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. ELISA 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. ELISA 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 vials 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 on 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, and 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 microtiter well 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.

SPECIMEN COLLECTION

Only 20 µL of human serum is required for human NSE measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg 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 - 8°C if the assay is to be performed within 24 hours. Otherwise, patient samples should be stored at -20°C or below until measurement. Avoid any repeated freezing and thawing of specimen.

Plasma sample is not recommended for NSE measurement.

ASSAY PROCEDURE

1. Reagent Preparation

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

- (2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
- (3) Reconstitute all assay standards (Cat. 30231-30235) and controls (Cat. 30236-30237) by adding **0.5 mL** of demineralized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is 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 (Cat. 10040) in a holder to run human NSE standards, controls and unknown samples in duplicate.
- (2) Test Configuration

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	
H	STD 4	SAMPLE 1	

- (3) Prepare NSE Tracer Antibody and Capture Antibody working solution by 1:21 fold dilution of the Tracer Antibody (Cat. 30239) with the biotinylated Capture Antibody (Cat. 30238). For each strip, it is required to 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 patient samples into the designated microwell.
- (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 ELISA HRP Substrate (Cat. 10020) 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 ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
- (12) Read the absorbance at 450 nm within 10 minutes in a microplate reader.
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, 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.

- Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
- Incubation times or temperatures other than those stated in this insert may affect the results.
- Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
- All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

- Calculate the average absorbance for each pair of duplicate test results.
- Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
- 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.

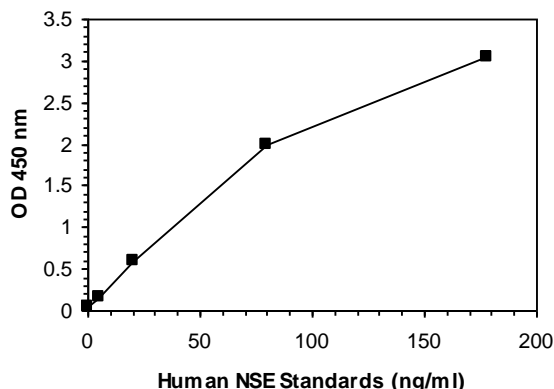
The human NSE concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

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 standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.042	0.043	0.000	
	0.043			
5 ng/mL	0.170	0.166	0.123	
	0.162			
20 ng/mL	0.597	0.592	0.549	
	0.587			
80 ng/mL	2.058	1.979	1.936	
	1.899			
178 ng/mL	3.040	3.040	2.997	
	3.040			
Control 1	0.359	0.359	0.316	11.27 ng/mL
	0.358			
Control 2	2.803	2.859	2.816	147.24 ng/mL
	2.914			

Human NSE ELISA



EXPECTED VALUES

One hundred seventy two normal adult sera were measured with this human NSE ELISA. The normal range was found to be less than 15 ng/mL. It is highly recommended that each laboratory should establish its own normal cut off level.

Although a serum NSE level above 15 ng/mL would be an aid in clinical diagnosis, it is recommended to establish a baseline level of NSE for each patient for monitoring cancer patients after surgery. A clear surge of NSE level would indicate an increased cancer cell activity.

LIMITATION OF THE PROCEDURE

- 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.
- For sample values reading greater than the highest standard, it is recommended to re-assay samples with dilution.
- Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
- Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

PERFORMANCE CHARACTERISTICS

Sensitivity

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

High Dose "hook" effect

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

Precision

The intra-assay precision is validated by measuring two controls 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	22.40	22.25	101
2	Neat	146	-	-
	1:2	71.44	73	98
	1:4	34.62	36.5	95

Recovery

Two patient 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

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.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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Short Assay Procedure:

1. Add 10 µL of standards, controls and patient serum samples into the designated microwell.
1. Add 100 µL of the mixture of tracer and capture antibodies to each well.
2. Mix, cover and incubate the plate at room temperature, shaking at 170 rpm for 1 hour.
3. Wash each well 5 times.
4. Add 100 µL of ELISA HRP Substrate into each of the wells.
5. Cover and incubate plate at room temperature for 10 minutes or less.
7. Add 100 µL of ELISA Stop Solution into each of the wells.
8. Read the absorbance at 450 nm.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE












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