EAGLE BIOSCIENCES

Neuron Specific Enolase (NSE) ELISA Kit

Catalog Number:
NSE31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 7 (effective 30JUN21)

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INTENDED USE

The Eagle Biosciences Neuron Specific Enolase ELISA Kit is intended for the quantitative determination of human neuron specific enolase (NSE) levels in serum. The Eagle Biosciences Neuron Specific Enolase (NSE) ELISA Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

The glycolytic enzyme enolase (2-phospho-Dglycerate hydrolyase) 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 initially were detected in neurons and neuroendocrine cells. This test detects both the αγ and the γγ 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 denign disease. Lung disease is one of the most common cancer forms with incidences about 50-100 per 100,000 population. Approximately 20% of the lunc 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.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Neuron Specific Enolase (NSE) ELISA Kit 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 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.

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LIMITATIONS RELATED TO INTENDED USE

- 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 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 inactive the horseradish peroxidase enzyme.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.5 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.

- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS
The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS
Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage
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. Samples should not be taken from individuals taking biotin-containing multivitamins or dietary supplements at least 48 hours 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, 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.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
- Precision single channel pipettes capable of delivering 10, 50, 100 and 1000 µL
- Repeating dispenser suitable for delivering 100 µL
- Disposable pipette tips suitable for above volume dispensing
- Disposable 12x75 mm or 13x100 glass or plastic tubes
- Disposable plastic 100 mL and 1000 mL bottle with caps

- Aluminum foil
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system
- Plate Shaker
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

REAGENTS PROVIDED

1. **Streptavidin Coated Microplate**

Contents:	One 96-well (12 x 8) microplate coated with streptavidin.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

2. **NSE Tracer Antibody (21x)**

Contents:	One bottle containing concentrated HRP-labeled anti-human NSE-specific monoclonal antibody in a stabilized protein matrix.
Format:	Concentrated; Requires Preparation
Volume:	0.6 mL/bottle
Storage:	2-8°C
Stability:	Concentrated: Stable until the expiry date printed on the label. After preparation: Discard after use.
Preparation of Working Solution:	**Dilute 1:21** with NSE Capture Antibody Diluent prior to use. 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. Prepare just before use.

3. **NSE Capture Antibody**

Contents:	One bottle of biotinylated NSE capture antibody. Should only be used after mixing with NSE tracer antibody.
Format:	Ready to Use
Volume:	12 mL
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label

4. **ELISA Wash Concentrate (30x)**

Contents:	One bottle of concentrated wash buffer containing a surfactant in phosphate buffered saline with non-azide, non-mercury based preservative.
Format:	Concentrated; Requires Preparation
Volume:	30 mL/bottle
Storage:	2-25°C
Stability:	Unopened: Stable until the expiry date printed on the label.
Preparation of Working Solution:	**Dilute 1:30.** Combine the contents with 870 mL of distilled or deionized water and mix well before use.

5. **ELISA HRP Substrate**

Contents:	One bottle containing tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

6. **ELISA Stop Solution**

Contents:	One bottle containing 0.5 M sulfuric acid
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-25°C
Stability:	Stable until the expiry date printed on the label.

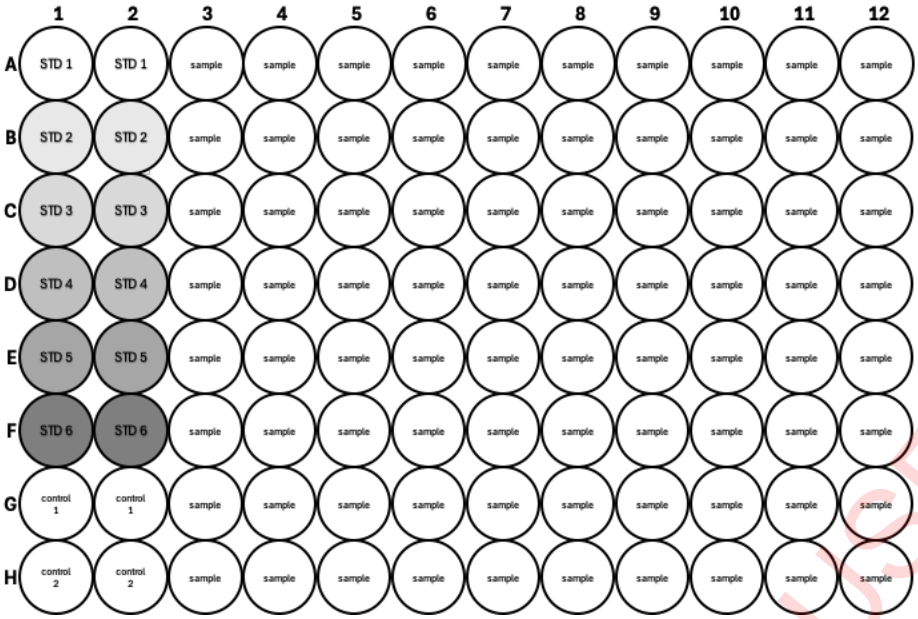
7. **NSE Standards**

Contents:	Two sets of five bottles each containing human NSE in lyophilized bovine serum-based matrix with a non-azide, non-mercury based preservative. Refer to bottles for exact concentration.
Format:	Lyophilized; Needs Reconstitution
Storage:	2-8°C
Stability	Unopened: Stable until the expiry date printed on the label. After Reconstitution: Stable for 30 days
Preparation of Working Solution:	Add 0.5 mL of distilled or deionized water, allow to sit for 10 minutes undisturbed, then mix well by inversions or gently vortexing. Make sure all solids are dissolved completely prior to use.

8. **NSE Controls**

Contents:	Two vials each containing human NSE in a lyophilized bovine serum-based matrix with a non-azide, non-mercury preservative. Refer to bottles for exact concentration.
Format:	Lyophilized; Needs Reconstitution
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Reconstitution: Stable for 30 days
Preparation of Working Solution.	Add 0.5 mL of distilled or deionized water, allow to sit for 10 minutes undisturbed, then mix well by inversions or gently vortexing. Make sure all solids are dissolved completely prior to use.

RECOMMENDED ASSAY LAYOUT*



*****Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Place a sufficient number of streptavidin coated microwell strips in a holder to run standards, controls and samples in duplicate.
2. Prepare NSE Tracer Antibody and Capture Antibody working solution by 1:21 fold dilution.
3. Add **10 µL of standards, controls and samples** into designated wells.
4. Add **100 µL** of above mixture of Trace Antibody and Capture Antibody solution to each of the wells.
5. Cover the plate with one plate sealer and incubate place at **room temperature,** with **shaking at 170 rpm (bigger radius) or 400 rpm (smaller radius)** for **1 hour.**
6. 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 aspirate the contents. Alternatively, an automated microplate washer can be used.
7. Add **100 µL** of HRP Substrate into each of the wells.
8. Cover the plate with one plate sealer and aluminum foil. Incubate plate at **room temperature** for **10 minutes** or less.
9. Remove the aluminum foil and plate sealer. Add **100 µL** of Stop Solution into each of the wells. Mix gently.
10. 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.

CALCULATIONS

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 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.
4. It is recommended to 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.

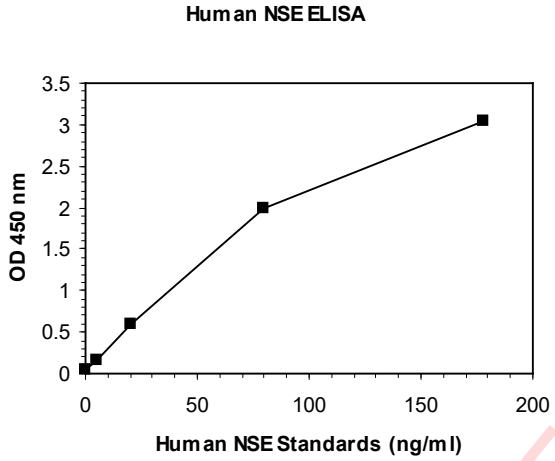
QUALITY CONTROL

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.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from the Neuron Specific Enolase (NSE) ELISA Kit 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	0.043	0.000	
5 ng/mL	0.170 0.162	0.166	0.123	
20 ng/mL	0.597 0.587	0.592	0.549	
80 ng/mL	2.058 1.899	1.979	1.936	
178 ng/mL	3.040 3.040	3.040	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



EXPECTED VALUES

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

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.

Intra-assay

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

Inter-assay

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 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 %
Sample 1	Sample 1	10.32	10.14	102
	Sample 2	20.78	21.68	96
Sample 2	Sample 3	10.07	10.23	98
	Sample 4	23.88	20.35	117

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WARRANTY INFORMATION

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