Human Neuron Specific Enolase (NSE) ELISA

Catalog # LF-EK0169 (1 kit bundle)

Catalog # LF-EK0170 (4 kits bundle)

Catalog # LF-EK0171 (10 kits bundle)

Catalog # LF-EK0172 (20 kits bundle)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of human Neuron Specific Enolase(NSE)

For research use only
Not for diagnostic or therapeutic procedures

Contents

1. Introduction

Enolase (2-phosphogly-cerate hydrolyase or phosphopyruvate hydrates) is a glycolytic enzyme that catalyzes the dehydration and conversion of 2-phosphoglycerate to phosphoenolpyruvate. It comprises three distint subunits, α, β and γ. The γγ and αγ dimeric forms of enolase, referred to as neuron-specific enolase(NSE), are localized mainly in neurons and neuroectodermal tissue. NSE has a high stability in biological fluids and can easily diffuse to the extracellular medium and cerebrospinal fluid(CSF) when neuronal membranes are injured. NSE is used clinically as a sensitive and useful marker of neuronal damage in several neurological disorders including stroke, hypoxic brain damage, status epilepticus, Creutzfeldt-Jakob disease, and herpetic encephalitis.

2. Principles of Method

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human NSE. Samples are pippetted into these wells. Nonbound NSE and other components of the sample should be removed by washing, then biotin-conjugated monoclonal antibody specific to NSE added. In order to quantitatively determine the amount of NSE present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) should be added to each microplate well. The final step, a TMB-substrate solution added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450nm. Since the increases in absorbency is directly proportional to the amount of captured NSE.

3. Intended Use

The AbFrontier human neuron specific enolase(human NSE) ELISA kit is to be used for the in vitro quantitative determination of human NSE in human serum, human plasma, cell lysate and buffered solution. The assay will recognize native NSE.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8℃. Any unused reconstituted standard should be discarded or frozen at -70℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Incubation Buffer	1	30ml
Washing Buffer	1	(10X) 100ml
Standard Protein	1 Glass vial (lyophilized)	
Standard/Sample Dilution Buffer	1	25ml
Secondary Antibody	1 Glass vial (lyophilized)	
AV-HRP	1	150ul
Secondary Antibody/AV-HRP Dilution Buffer	1	25ml
Substrate (TMB)	1	20ml
Stop Solution	1	20ml
Protocol booklet	1	
Plate sealers	2	

① 96 Well Plate

: Human NSE microtiter plate, one plate of 96 wells (16well strip x 6).

A plate using break-apart strips coated with a mouse monoclonal antibody specific to human neuron specific enolase.

② Standard Protein

: Native human neuron specific enolase.

③ Secondary Antibody

: Biotin labeled mouse anti human neuron specific enolase antibody.

④ AV-HRP

: Avidin linked Horseradish Peroxidase (HRP, enzyme)

⑤ Substrate (Stabilized chromogen)

: Tetramethylbenzidine (TMB) solution

⑥ Stop Solution

: 1N solution of sulphuric acid (H_2SO_4).

⑦ Plate sealer

: Adhesive sheet.

- Do not mix or interchange different reagents from various kit lots.

7. Materials Required But Not Provided

① Microtiter plate reader capable of measurement at or near 450nm.
② Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
③ Distilled or deionized water
④ Data analysis and graphing software
⑤ Vortex mixer
⑥ Polypropylene tubes for diluting and aliquoting standard
⑦ Absorbent paper towels
⑧ Calibrated beakers and graduated cylinders of various sizes

8. Reagent Preparation

1) Human Neuron specific enolase standard

Reconstitute the human NSE standard to 0.5ug/ml by adding 1ml of *Standard/Sample Dilution Buffer* into the standard protein glass vial containing lyophilized human NSE protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

Standard	Add	Into
80ng/ml	160ul of the std.(0.5ug/ml)	840ul of the *Standard/Sample Dilution Buffer*
40ng/ml	80ul of the std.(0.5ug/ml)	920ul of the *Standard/Sample Dilution Buffer*
20ng/ml	40ul of the std.(0.5ug/ml)	960ul of the *Standard/Sample Dilution Buffer*
10ng/ml	20ul of the std.(0.5ug/ml)	980ul of the *Standard/Sample Dilution Buffer*
5ng/ml	10ul of the std.(0.5ug/ml)	990ul of the *Standard/Sample Dilution Buffer*
2.5ng/ml	5ul of the std.(0.5ug/ml)	995ul of the *Standard/Sample Dilution Buffer*
1.25ng/ml	2.5ul of the std.(0.5ug/ml)	997.5ul of the *Standard/Sample Dilution Buffer*
0ng/ml	1.0ml of the *Standard/Sample Dilution Buffer*	

2) Secondary Antibody

100X secondary antibody solution can be made by adding 150 $\mu\ell$ secondary antibody/AV-HRP dilution buffer in the vial.

1. Equilibrate to room temperature, mix gently.
2. Mix 20ul *Secondary Antibody concentrated solution* (100X) + 2ml *Secondary Antibody/AV-HRP dilution buffer*. (Sufficient for one 16-well strip, prepare more if necessary)

Label as “Working Secondary antibody Solution”.

3. Return the unused *Secondary Antibody concentrated solution* to the refrigerator.

3) AV-HRP

1. Equilibrate to room temperature, mix gently.
2. Mix 20ul *AV-HRP concentrated solution* (100X) + 2ml *Secondary Antibody/AV-HRP dilution buffer*. (Sufficient for one 16-well strip, prepare more if needed)
   Label as “Working AV-HRP Solution”.
3. Return the unused *AV-HRP concentrated solution* to the refrigerator.

4) Washing buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 1 volume *Wash buffer concentrate solution* (10X) + 9 volumes of deionized water.
   Label as “Working Washing Solution”.
3. Store both the concentrated and the Working Washing Solution in the refrigerator.

*** Directions for washing**

1. Fill the wells with 300ul of “Working Washing Buffer”.
   Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.
   If using an automated washer, the operating instructions for washing equipment should be carefully followed.
2. Incomplete washing will adversary affects the assay and renders false results.
3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

5) Sample preparation

Blood should be collected by venipuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8℃ if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20℃. Avoid repeated freeze/thawing.

9. Assay Procedure

- Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
- All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- A standard curve must be run with each assay.
- If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.

1) Determine the number of 16-well strips needed for assay. Insert these in the flame(s) for current use (Re-bag extra strips and frame. Refrigerate for further use).
2) Add 300ul of *Incubation buffer* to all wells and incubate the plate for 5 minutes at room temperature.
3) Thoroughly aspirate or decant the solution from the wells. Wash wells 2 times (See "Directions for washing").
4) For the standard curve, add 100ul of the standard to the appropriate microtiter wells. Add 100ul of the *Standard/Sample Dilution Buffer* to zero wells.
5) Serum and plasma require **at least 20 fold dilution** in the *Standard/Sample Dilution Buffer.* And add 100ul of samples to each wells.
6) Cover the plate with the plate cover and incubate for 2 hours at 37℃.
7) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
8) Pipette 100ul of "Working Secondary Antibody Solution" into each well.
9) Cover the plate with the plate cover and incubate for 1 hour at room temperature.
10) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
11) Add 100ul "Working AV-HRP Solution" to each well.
12) Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
13) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
14) Add 100ul of *Substrate* to each well. The liquid in the wells should begin to turn blue.
15) Incubate the plate at room temperature.

- Do not cover the plate with aluminum foil, or color may develop.

  The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before

O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450nm can only be read after the Stop Solution has been added to each well.

- Because the *Substrate* is light sensitive, avoid the remained *Substrate* solution prolonged exposure to light.
- Typically, reaction is stopped 5~10 minutes after treatment of Substrate, but this time can be adjusted as the user desires.

16) Add 100ul of *Stop Solution* to each well. The solution in the wells should change from blue to yellow.

17) Read the absorbance of each well at 450nm. Read the plate within 20 minutes of adding the *Stop Solution*.

18) Plot on graph paper the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.

19) Read the human NSE concentrations for the unknown samples and controls from the standard curve plotted in step 18. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest standard should be further diluted in the S*tandard/Sample Dilution Buffer*).

10. Characteristics

1) Typical result

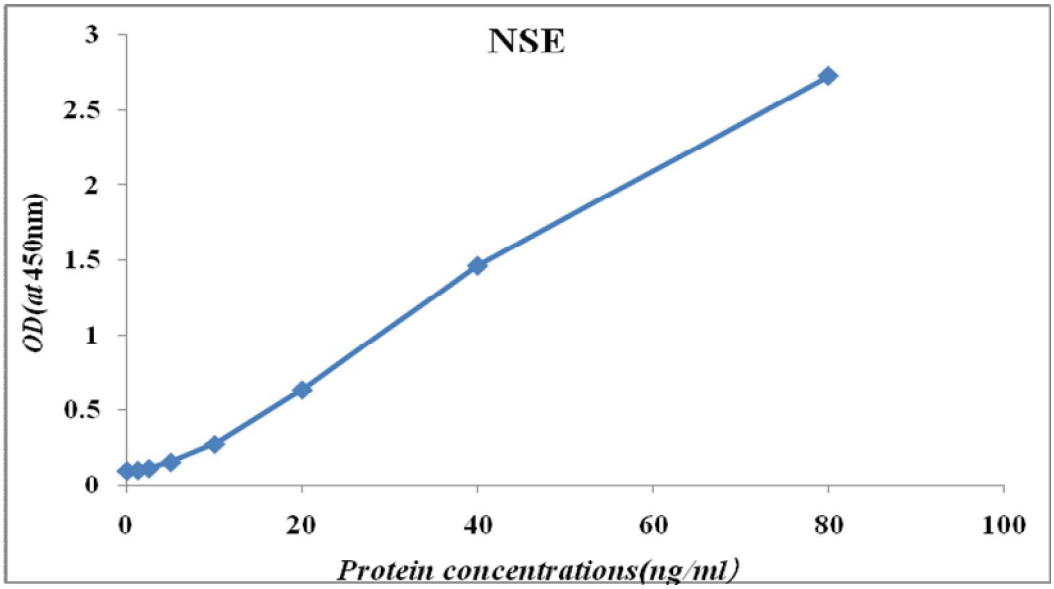
The standard curve below is for illustration only and **should not be used** to calculate results in your assay.

A standard curve must be run with each assay.

Standard human NSE (ng/ml)	Optical Density (at 450nm)
0	**0.093**
1.25	**0.097**
2.5	**0.109**
5	**0.151**
10	**0.273**
20	**0.634**
40	**1.463**
80	**2.728**

< Limitations >

- Do not extrapolate the standard curve beyond the 80ng/ml standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native human neuron specific enolase in various matrices has not been investigated.



(TMB reaction incubate at room temperature for 5min)

2) Sensitivity

The minimal detectable dose of human NSE was calculated to be 0.15ng/ml, by subtracting two standard deviations from the mean of 12 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

N	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
ZERO	0.09	0.088	0.089	0.091	0.09	0.096	0.093	0.102	0.096	0.094	0.094	0.097

Average	SD	LLD	LLD mean(ng/ml)
0.093	0.00403	0.101394	2.447

3) Specificity

The following substances were tested and found to have no cross-reactivity: human serum albumin, human non neuronal enolase, human alpha fetoprotein, human prostate specific antigen(PSA), human hemoglobin, human VDBP(vitamin D binding protein)

4) Precision

① Within-Run (Intra-Assay)

(n=6)

Mean (ng/ml)	SD (ng/ml)	CV (%)
7.44	0.26	3.47
17.46	0.84	4.81
43.03	2.22	5.17
79.58	3.41	4.28

② Between-Run (Inter-Assay)

(n=4)

Mean (ng/ml)	SD (ng/ml)	CV (%)
7.15	0.43	6.07
17.31	0.74	4.25
42.87	2.20	5.13
79.43	3.47	4.37

5) Recovery

Recovery on addition is 75.36~108.38% (mean 91.94%)

Analyte addded (ng/ml)	Serum A (ng/ml)	Recovery (%)
10.0	7.64	86.03
20.0	14.44	75.36
40.0	34.44	89.90
80.0	86.80	108.38

11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30℃ before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12.Reference

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3. Suzuki Y. et al. (1999) Neurology 53(8), 1761-1764

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