ENO2 (Human) ELISA Kit

Catalog Number KA2122

96 assays

Version: 04

Intended for research use only

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Table of Contents

Introduction

Intended Use

The ENO2 (Human) 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.

Background

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.

Principle of the Assay

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 pipetted 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 450 nm. Since the increases in absorbency is directly proportional to the amount of captured NSE.

General Information

Materials Supplied

List of component

Component	Amount
Human NSE microtiter plate: A plate using break-apart strips coated with a mouse monoclonal antibody specific to human neuron specific enolase.	96 (8x 12) wells
Incubation Buffer	30 mL
Washing Buffer (20x)	25 mL x 2
Standard Protein (lyophilized): Native human neuron specific enolase.	1 vial
Standard/Sample Dilution Buffer	25 mL
Secondary Antibody (lyophilized): Biotinylated anti human neuron specific enolase antibody.	1 vial
AV-HRP: Avidin linked Horseradish Peroxidase (HRP, enzyme)	150 µL
Secondary Antibody/AV-HRP Dilution Buffer	25 mL
Substrate (Stabilized chromogen): Tetramethylbenzidine (TMB) solution	15 mL
Stop Solution: 1 N solution of sulfuric acid (H_2SO_4)	15 mL
Plate sealers: Adhesive sheet.	2 slices

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

Storage Instruction

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

Materials Required but Not Supplied

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

Precautions for Use

- ✓ 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.
- ✓ Standard protein and 2nd Antibody containing Sodium Azide as a preservative.

Assay Protocol

Reagent Preparation

- Human Neuron specific enolase standard

1. Reconstitute the lyophilized Human NSE standard by adding 1 mL of Standard/Sample Dilution Buffer to make the 0.5 µg/mL standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting
2. Prepare 1 mL of 80 ng/mL top standard by adding 160 µL of the above stock solution in 840 µL of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (1.25 ng/mL ~ 80 ng/mL) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 ng/mL).

Standard	Add	Into
80 ng/mL	160 µL of the std.(0.5 ug/mL)	840 µL of the Standard/Sample Dilution Buffer
40 ng/mL	500 µL of the std.(80 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
20 ng/mL	500 µL of the std.(40 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
10 ng/mL	500 µL of the std.(20 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
5 ng/mL	500 µL of the std.(10 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
2.5 ng/mL	500 µL of the std.(5 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
1.25 ng/mL	500 µL of the std.(2.5 ng/mL)	500 µL of the Standard/Sample Dilution Buffer
0 ng/mL	1.0 mL of the Standard/Sample Dilution Buffer	

- Secondary Antibody

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

1. Equilibrate to room temperature, mix gently.
2. Mix 20 µL Secondary Antibody concentrated solution (100X) + 1.98 mL Secondary Antibody/AV-HRP dilution buffer. (Sufficient for two 8-well strip, prepare more if necessary) Label as “Working Secondary antibody Solution”.
3. Return the unused Secondary Antibody concentrated solution to the refrigerator.

- AV-HRP

1. Equilibrate to room temperature, mix gently.
2. Mix 20 µL AV-HRP concentrated solution (100X) + 1.98 mL Secondary Antibody/AV-HRP dilution buffer. (Sufficient for two 8-well strip, prepare more if needed) Label as “Working AV-HRP Solution”.
3. Return the unused AV-HRP concentrated solution to the refrigerator.

- Washing buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 0.5 volume Wash buffer concentrate solution (20X) + 9.5 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 300 µL 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.*

Sample Preparation

Blood should be collected by veinpuncture. 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°C 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°C. Avoid repeated freeze/thawing.

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 300 µL 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 100 µL of the standard to the appropriate microtiter wells. Add 100 µL 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 100 µL of samples to each wells.
6. Cover the plate with the plate cover and incubate for 2 hours at 37°C.
7. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
8. Pipette 100 µL 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 100 µL "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 100 µL 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 450 nm 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 100 µL 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 450 nm. 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 Standard/Sample Dilution Buffer).

Data Analysis

Calculation of Results

- 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.

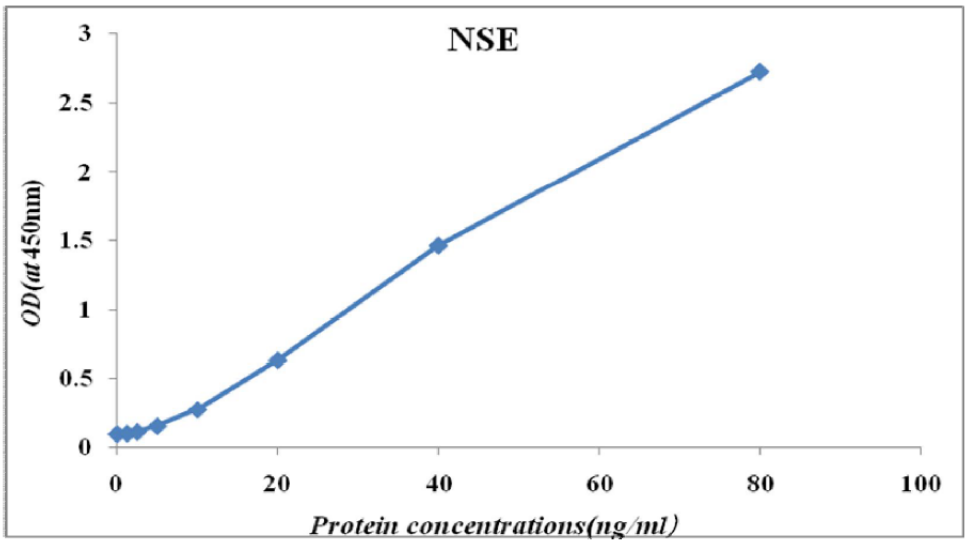
Standard Human NSE (ng/mL)	Optical Density (at 450 nm)
0	0.093
1.25	0.097
2.5	0.109
5	0.151
10	0.273
20	0.634
40	1.463
80	2.728

- Limitation

✓ Do not extrapolate the standard curve beyond the 80 ng/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)

Performance Characteristics

- Sensitivity

  The minimal detectable dose of human NSE was calculated to be 0.15 ng/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

- 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)

- Precision

  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

  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

- Recovery

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

Analyte added (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

Resources

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°C 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

References

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2. Lima J.E. et al. (2004) J. Neurol. Sci. 217(1), 31-35
3. Suzuki Y. et al. (1999) Neurology 53(8), 1761-1764

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H