Human NSE ELISA Kit

Enzyme Immunoassay for the quantification of Human NSE in human serum samples.

Catalog number: ARG80756

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INTRODUCTION

Neuron specific enolase (NSE), also known as enolase 2 (ENO2) or Gamma-enolase, is an enzyme that in humans is encoded by the ENO2 gene. NSE is a phosphopyruvate hydratase.

NSE is one of the three enolase isoenzymes found in mammals. This isoenzyme, a homodimer, is found in mature neurons and cells of neuronal origin. A switch from alpha enolase to gamma enolase occurs in neural tissue during development in rats and primates.

Detection of NSE with antibodies can be used to identify neuronal cells and cells with neuroendocrine differentiation. NSE is produced by small-cell carcinomas, which are neuroendocrine in origin. NSE is therefore a useful tumor marker for distinguishing small-cell carcinomas from other tumors. [Provide by Wikipedia: Enolase 2]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique.

A capture antibody specific for Human NSE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Human NSE present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-conjugated antibody specific for Human NSE is added to each well and incubate. After washing away any unbound substances, the substrate solution (TMB substrate) is added to the wells and color develops in proportion to the amount of Human NSE bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm.The concentration of Human NSE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C in the dark. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standards 0-4 (0, 4, 20, 50, 100 ng/ml)	2 vials each (lyophilized)	4°C
Control 1 (Negative control)	2 vials (lyophilized)	4°C
Control 2 (Positive control)	2 vials (lyophilized)	4°C
Diluent Buffer	50 ml (ready to use)	4°C
HRP-conjugated anti-Human NSE antibody	1 ml	4°C
50X Washing Buffer	20 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620-630 nm as optional reference wave length)
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C in dark at all times.
- Open the bag of Antibody-Coated Microplate only when it is at room temperature and close it immediately after use; once opened, the plate is stable up to expiry date.
- The reconstituted Standard and controls should be aliquoted before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles.
- Allow all kit components and specimens to reach room temperature (22°C-28 °C) and mix well prior to use.
- Briefly spin down the all vials before use.
- If crystals are observed in the 50X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not

become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note:

1. The serum would have to be separated from the blood within 60 minutes in order to avoid the increment of the human NSE from the blood cells release.
2. Do not use haemolytic, icteric or lipaemic specimens.
3. Avoid use of plasma since meaningful amounts of human NSE could be yielded from platelets.
4. Do not allow the samples at room temperature for long period.
5. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 10 ml of 50X Wash buffer into 490 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks when stored at 2-8°C.
- HRP-conjugated anti-Human NSE antibody : Prepare immediately before use. Add 20 µL of HRP-conjugated anti-Human NSE antibody to 1 mL of Diluent buffer, the quantity to prepare is directly proportional to the number of tests. Mix gently leaving in a rotating shaker for at least 5 minutes.
- Standards and control: Reconstitute each vial of standard and control with 0.75 mL of distilled water before use. Leave on a rolling mixer for about 5 minutes. Make sure the standards and controls are dissolved completely. Aliquot the reconstituted standards and controls. Take the necessary aliquot for the assay and immediately store unused standards and controls at-20°C for up to one month. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-28°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µL** of **standards, controls and samples** into the appropriate wells of Antibody-coated microplate.
3. Add **100 µL** of the **diluted HRP-conjugated anti-Human NSE antibody** to each well.
4. Cover the plate and incubate for **1 hours** at **RT** (22-28°C).
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Gently shake the plate for 5 seconds. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. (For automated equipment is used, wash the wells at least 5 times.)
6. Add **100 µl** of **TMB Substrate** to each well. Incubate for **15 minutes at room temperature** in the dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
8. Read the OD with a microplate reader at **450 nm** immediately. (optional:

read at 620-630 nm as reference wavelength) It is recommended read the absorbance within 5 minutes after adding the stop solution.

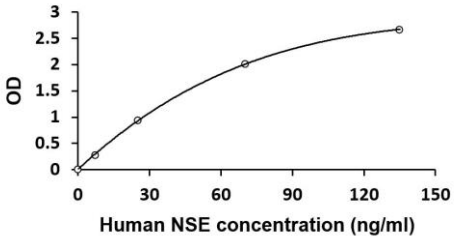
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
7. Reference values

	Human NSE
Normal range	0-12 ng/mL
Pathological value	>12 ng/mL

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Human LOX-1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

The lowest detectable concentration of human NSE is 0.19 ng/ml

Specificity

The antibody is directed specifically against the human neuron specific enolase.

Cross reactivity values have been calculated on a weight/weight basis.

NSE 100%

NNE <0.22%

Intra-assay and Inter-assay precision

The CV value of intra-assay was ≤4.4% and inter-assay precision was ≤ 11.2%.