

Product Information

Neuron Specific Enolase (NSE) ELISA

Catalog Number **SE120098**

TECHNICAL BULLETIN

Product Description

Neuron specific enolase (NSE) is a neuronal form of the glycolytic enzyme enolase, which was first found in extracts of brain tissue, and was later shown to be present in APUD (amine precursor uptake and decarboxylation) cells and neurons of the diffuse neuroendocrine system but not in other peripheral cells.

This glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\alpha\gamma$, $\beta\beta$, and $\gamma\gamma$) composed of three distinct subunits α , β , and γ . Three isoenzymes are found in human brain: $\alpha\alpha$, $\alpha\gamma$, and $\gamma\gamma$. The γ unit is found either in a homologous $\gamma\gamma$ or in a heterologous $\alpha\gamma$ -isoenzyme, and is known as neuron-specific enolase (NSE). NSE is a valuable tumor marker for cancers of neuroendocrine origin, especially for small-cell lung cancer and neuroblastoma.

The Neuron Specific Enolase (NSE) ELISA (enzyme-linked immunosorbent assay) is intended for the quantitative determination of NSE levels in human serum. The NSE kit is a solid phase direct sandwich ELISA method. The standards, samples, controls are added into the selected wells pre-coated with anti-human NSE monoclonal antibody along with the anti-NSE-HRP conjugated pair match antibody. NSE, in the standards, controls, and samples binds to anti-NSE antibody on the wells and anti-NSE-HRP conjugated antibody binds to the NSE. The unbound glycolytic enzyme enolase, NSE, is washed off by wash buffer. Upon the addition of the TMB substrate, the intensity of color developed is proportional to the concentration of NSE in the samples. A standard curve is prepared relating color intensity to the concentration of the NSE.

Components

Materials Provided	96 Tests
Microwells coated with Anti-NSE MAb	12 x 8 x 1
NSE Standard: 6 vials; Frozen	0.25 mL
NSE Enzyme Conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 72 hours. If storage time exceeds 72 hours, store frozen at (–20 °C or lower) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20x Wash Buffer Concentrate

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the NSE Standard at –70 °C and the rest of the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µL of NSE standards, control, and sera into selected wells.
3. Add 100 µL of working solution of anti-NSE enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C) with shaking (600 rpm).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash buffer. Blot on absorbent paper towels.
6. Add 100 µL of TMB Substrate to all wells.
7. Incubate for 30 minutes at room temperature.
8. Add 50 µL of Stop Solution to all wells. Shake the plate gently, for 10 seconds, to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Results**Calculations**

The standard curve is constructed as follows:

1. Check NSE standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the NSE standards (vertical axis) versus the NSE standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Example of a standard curve

	Concentration (ng/ mL)	OD 450 nm
Std 1	0	0.008
Std 2	5	0.069
Std 3	15	0.198
Std 4	35	0.500
Std 5	75	1.105
Std 6	150	2.130

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

References

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2. Paus, E., and Nustad, K., Immunoradiometric Assay for $\alpha\gamma$ - and $\gamma\gamma$ -Enolase (Neuron-Specific Enolase), with Use of Monoclonal Antibodies and Magnetizable Polymer Particles. *Clin. Chem.*, 1989, 35: 2034-2038.
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5. Elisabeth, p., and Kjell, N., Immunoradiometric assay for the $\alpha\gamma$ and $\gamma\gamma$ -enolase (Neurons-Specific Enolase), with use of monoclonal antibodies and magnetizable polymer particles. *Clin. Chem.*, 1989, 35/10: 2034-2038

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