NOVATEINBIO
Accelerates Research and Development

Rat Neuron Specific Enolase
NSE ELISA Kit

Catalog number: BG-RAT11620 (96 wells)

The kit is designed to quantitatively detect the levels of Rat NSE in
cell culture supernatants, cell lysates, serum, and plasma.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- **The operation should be carried out in strict accordance with the provided instruction.**
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 20 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

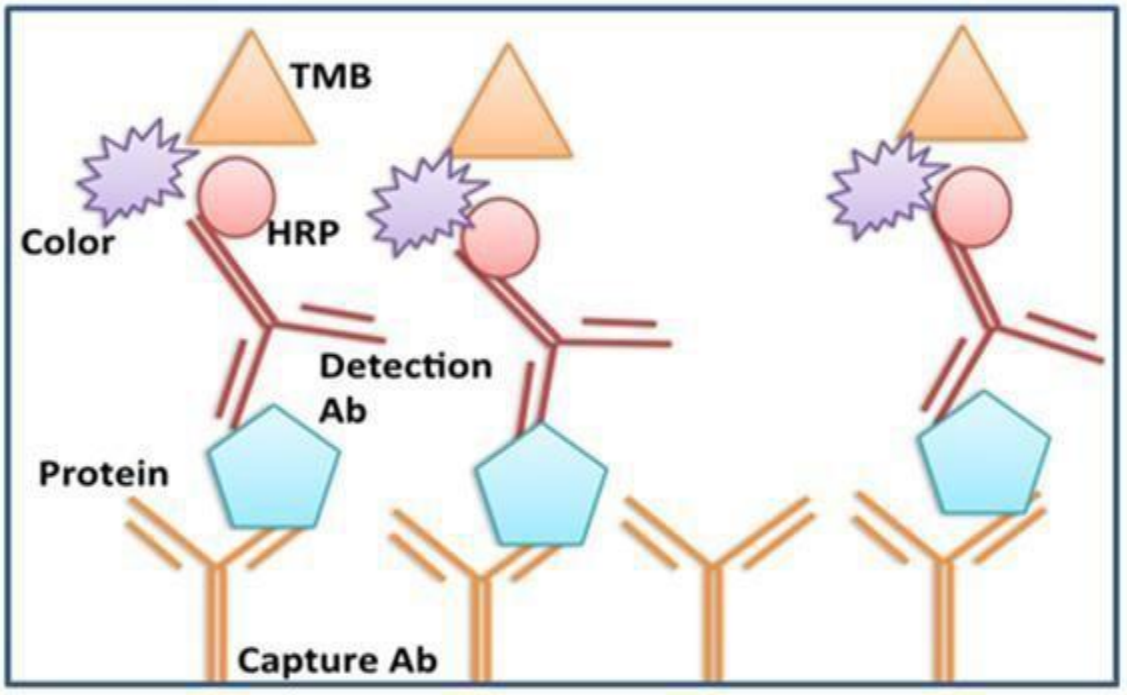
Intended use

The kit is used to quantify the Rat NSE in cell culture supernatants, cell lysates, serum, and plasma.

Standard range	78.1-5,000 pg/ml
Sensitivity	10 pg/ml
Assay time	5 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Rat NSE ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Rat NSE specific antibody has been precoated onto 96-well plate. The test samples, detection antibody-biotin, and the Streptavidin-HRP are added to the wells subsequently and then followed by plate-well washing. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow color is proportional to the Rat NSE amount captured from the sample.



Materials supplied

1. Rat NSE standard:	3	ng/vial x1
2. 96-well plate coated with anti-Rat NSE Ab:		1.
3. Assay Diluent buffer :		12 ml x 4.
4. Detection antibody:		1 vial.
5. Streptavidin-HRP		1 vial.
6. Chromogen solution A:		6 ml.
7. Chromogen solution B:		6 ml.
8. Stop Solution:		6 ml.
9. 20 × Wash Buffer:		25 ml.
10. Plate sealer		1.
11. Package insert		1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

- CSF, tissue lysate or other tissue lysate or body fluid: Remove particulates by centrifugation at 3000 x g for 10 minutes, analyze immediately or aliquot and store at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. **The user should determine the optimal dilution factor**.
- Cell lysates: Remove culture media by centrifugation at 2000 x g for 10 minutes. Wash cells once with cold PBS. Pour off any excess PBS. For a T75 flask, add 10 ml of Cell Lysis Buffer (1 mM EDTA, 0.5% Triton X-100, 10 g/mL Leupeptin, 10 g/mL Pepstatin, 3 g/mL

Aprotinin, 150 mM NaCl, 10 mM NaF, 20 mM , β- glycerophosphate in PBS, pH 7.2-7.4) and incubate at room temperature for one hour with gentle agitation. Then collect lysates and centrifuge at 12,000 x g for 10 minutes to remove insoluble cell debris. The total protein concentration of the lysate supernatants may be determined by the Bradford method.

- Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before
  centrifugation for 15 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
- Plasma: Collect plasma on ice using heparin as an anticoagulant. Centrifuge for 15 minutes at 2-8° C at 2000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8° C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note:

- NSE is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of NSE, platelet-free plasma should be collected for measurement.
- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- This kit is designed to detect Rat NSE in cell culture supernatants, serum, plasma and other suitable sample solution. The users should check the expression profile of this protein before using this kit. If the samples are not specifically indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. We recommend that a serum and/or plasma sample as the positive control should be run in each assay.

- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Can't detect the samples containing NaN3, since NaN3 inhibits HRP (horseradish peroxidase) activity.
- Sample hemolysis will influence the result, so hemolytic specimen cannot be detected. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation.

Standard

- Rat NSE: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (5 ng /vial) are included in each kit. Use one tube for each experiment.
- 5,000 pg/ml→78.1 pg/ml of Rat NSE standard solutions:
- Add 1 ml of Assay Diluent buffer into one standard tube with 5 ng Rat NSE. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 5,000 pg/ml standard solution.

  Label 6 Eppendorf tubes with 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.3 pg/ml, 78.1 pg/ml, respectively. Aliquot 0.5 ml of 5,000 pg/ml standard solution and 0.5 ml of Assay Diluent buffer into 2500 pg/ml tube, mix well. This is 2500 pg/ml standard solution. Then make 2-fold serial dilution from 2500 pg/ml to 78.1 pg/ml in six 1.5 ml tubes.
- Make sure each tube has ≥ 300 µl of standard.

  Note: The standard solutions are best used within 2 hours.

Detection Antibody

- The stock solution is stable at 2-8 ^{o}C for up to 1 month. After opening the vial use within 1 month. For long-term storage, please aliquot and store at -20 ^{o}C. Avoid freeze-thaw cycles.
- The working solution should be prepared no more than 2 hours prior to the experiment
- The reagent is supplied as 60X concentrate. Empty the total contents in to 11.8 ml of Assay Diluent Buffer or prepare the solution separately in a volume as needed. The solution should be mixed thoroughly.

- The total volume should be: 0.1 ml/well x the number of wells (Allowing 0.3-0.4 ml more than total volume).

Streptavidin-HRP

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted 1:40 with Assay Diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Rat NSE Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Rat NSE concentration in samples. We recommend that each Rat NSE standard solution and each sample is measured in duplicate.

1. Add 100 µl of sample or standards per well. Add 0.1 ml of the Assay diluent into the control well (Zero well). Cover with an adhesive strip and incubate at room temperature for 2 hours.
   Note: We recommend that each Rat NSE standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate at room temperature for 2 hours.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate at room temperature for 20 min. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for three times.
7. Add 100 µl of TMB substrate solution to each well. Cover and incubate at room temperature for 5 -25 min or until a gradient develops and you see visible color in the 2nd lowest concentration well. Protect from light. Do not over-develop.
8. Add 50 µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

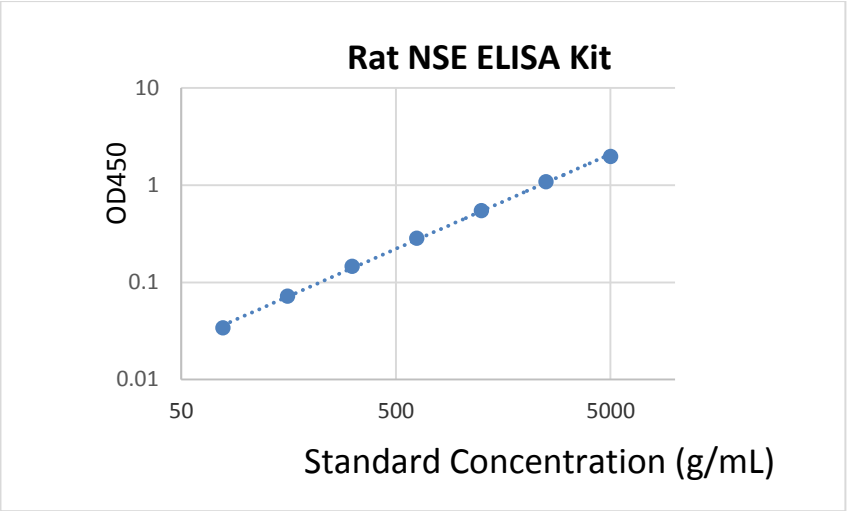
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat NSE concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical data

This standard curve was generated at Novatein biolab for demonstration purpose only. A standard curve must be run with each assay.



Background

Enolase (2-phospho-Dglycerate hydrolase) is a cytoplasmic enzyme that is responsible for converting 2-phosphoglycerate to phosphoenolpyruvate. Three members: Enolase 1, Enolase 2, and Enolase 3, which are also termed α, γ, and β enolase, respectively. They exist as several dimeric isoenzymes including αα, αβ, ββ, αγ, and γγ. The αα and γγ isoenzymes are abundant in neurons and neuroendocrine cells, and therefore, they are also designated as neuron specific enolase (NSE). Human Enolase 2 is 434 amino acids (aa) in length. It shares 83% aa identity with human enolases 1 and 3 and 99% with its mouse orthologue. Studies have shown that a variety of neurologic disorders such as stroke, traumatic brain injury, multiple sclerosis, and Alzheimer's disease initiated release of Enolase 2 from injured cells and elevated serum levels of Enolase 2.

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