Instruction for Use

【Product Name】

Neuronen-Spezifische Enolase (eCLIA)

【Order Information】

REF NO.	Package Size
0320501601	50 T
0320501602	2×50 T
0320501603	100 T
0320501604	2×100 T

【Intended Use】

Immunoassay for in vitro quantify determination of Neuronen-Spezifische Enolase (NSE) in human serum, which is clinically used for the treatment of lung cancer and neuroblastoma and the monitoring of recurrence.

Glycolenolase (2-Phospho-D-Glycerate Hydrolase.EC 4.2.4.11, molecular weight about 80kD) has a variety of dimeric isomers and consists of three subunits α, β, and γ.The α subunit of enolase is found in many types of mammalian tissues, while the β subunit is mainly found in heart and muscle tissues.There are five isomers in nature (αα, ββ, γγ, αβ, αγ), among which αγ and γγase isomers are called neuron-specific enolase (NSE) or γ-enzyme, which are the key enzymes that catalyze the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the TCA cycle of neurons and neuronal endocrine cells.The nucleotide sequence of the gene was 2423bp, the molecular weight was 80 kD, and the isoelectric point was pH 4.7. It was an acid proteinase[1].

Serum NSE is a kind of acidic protease peculiar to neurons and neuroendocrine cells, which exists in high concentration in nerve cells and neuroendocrine cells and in tumor cells induced by these cells.NSE is a specific marker of neuroendocrine tumors, which is mainly used in the differential diagnosis, disease monitoring, efficacy evaluation and recurrence prediction of patients with small cell lung cancer and neuroblastoma. It also has certain reference value in the differential diagnosis of other tumor diseases[2-4].

【Principles of the testing method】

Sandwich principle. Total duration of assay: 9 minutes.

20 μL of sample, biotinylated NSE monoclonal antibody and the ruthenium (Ru) complex-labeled NSE monoclonal antibody form an antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of NSE in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin coated magnetic particles, 0.45 mg/mL; 0.1 M phosphate-buffered saline (PBS); ProClin300	2×1.5 mL	1×1.5 mL	1×3.0 mL	2×3.0 mL
(RB)	Biotinylated NSE antibody, 1.0 mg/L; 0.1 M PBS; ProClin300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
(RA)	Ru complex- labeled NSE antibody, 1.0 mg/L; 0.1 M PBS; ProClin300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
Calibrators	NSE antigen, equinum serum; ProClin 300;lyophilized	High: 1×2.0 mL Low: 1×2.0 mL			
Control Materials (Optional)	NSE antigen, equinum serum; ProClin 300;lyophilized	High: 1×2.0 mL Low: 1×2.0 mL			

Traceability:This method can be traceable to Roche Elecsys NSE.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2℃-8℃.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened store at 2℃~8℃	18 months
Store at 2℃~8℃ after opening	8 weeks
Store on the analyzers at 2℃~15℃	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened store at 2℃~8℃	18 months
Store at 2℃~8℃ after resolution	3 days
Store at -25℃~-15℃ after resolution	2 months (freeze only twice)

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, preparation and storage】

Serum samples should be collected in standard test tubes or vacuum tubes with separation gel. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances, and the centrifugation time should not exceed 1 hour. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples. Do not use plasma.

Samples can be stored for 6 hours at room temperature (18~25℃), 24 hours at 2~8℃, and 3 months at -15℃ or below. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after one freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. NSE testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30℃; relative humidity: ≤80%.

Pre-treatment of freeze-dried calibrators and quality control products:

2.0 mL of deionized water was added accurately, and the contents of the bottle were dissolved by vertically standing with cap for 20 minutes at room temperature.Mix well to avoid bubbles.The mixed calibration products and quality control products shall be separated and marked.If not tested immediately, it should be quickly transferred to -15℃ or below -15℃ for storage.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended as follows :

(1) When using a new lot of reagent;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) If the quality control result exceeds the defined limit;
(4) When changing the buffers of different lot numbers.

Quality Control procedure

For quality control, use NSE control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used.

Calculation

The system software can automatically calculate the analyte concentration, with the result in ng/mLor μg/L.

1 ng/mL = 1 μg/L.

Specimen dilution

NSE concentration in the sample higher than the upper limit of detection can be diluted with sample diluent.

The recommended dilution ratio is 1:2 (automatic dilution by instrument or manual dilution). If manual dilution, the result should be multiplied by the dilution ratio. If instrument automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Interval】

By analyzing the serum samples of 171 healthy subjects from hospitals, it was calculated that the upper limit of the reference range of 95% is 16.25 ng/mL.

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 0.06~370 ng/mL. If the NSE concentration in the sample is lower than the lower limit of detection, the result is reported as <0.06 ng/mL; if the NSE concentration in the sample is higher than the upper limit of detection, the result is reported as >370 ng/mL (the 2-fold diluted sample is reported as > 740 ng/mL).

When jaundice (bilirubin) is ≤ 72 mg/dL, lipemia (triglyceride) is ≤ 2000 mg/dL, biotin is ≤ 70 ng/mL, total protein is ≤ 7 g/dL, and HAMA is ≤ 100 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is ≤ 1500 IU/mL, the relative recovery of the test results is between 90% and 110%.

When the sample contains adriamycin (100 μg/mL), methotrexate (500 μg/mL), cyclophosphamide (500 μg/mL), 5-fluorouracil (100 μg/mL), aspirin (500 μg/mL), Cischloroplatin (1000 μg/mL), Acetulolenic acid (200 μg/mL), the interference deviation of the measured results is within ±10%.

When the NSE concentration reaches 100000 ng/mL, the test results are not be affected by the hook effect.

【Analytical Performance】

Limit of Detection

Limit of Blank = 0.06 ng/mL.

Limit of Detection = 0.1ng/mL.

Accuracy

The accuracy control subject to standardized traceability is tested, and the relative deviation of its test results is within ± 10.0%.

Linearity

Within the range of 0.1 ng/mL~370 ng/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 6.0%.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10.0%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: coefficient of variation (CV) ≤ 6.0%.

Between-bottle homogeneity: coefficient of variation (CV) ≤ 10.0%.

Measured values of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range.

Analytical specificity

Test results of blank samples spiked with analogs below are lower than 0.06 ng/mL.

Analog	Concentration (ng/mL)
NNE	500
CYFRA21-1	100

Method comparison

A comparison of the Lifotronic NSE assay (y) with the Roche Elecsys NSE method (x) using clinical samples gave the following correlations:

Number of serum samples measured: 140

Linear regression:

Y=0.9345X+0.6579

r=0.9923

The sample concentrations were between 1.01 and351.9 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【References】

1. Lamerz R. NSE (Neuronen-spezifische Enolase), γ-Enolase. In: Thomas L (ed.). Clinical Laboratory Diagnosis, TH-Books, Frankfurt, 1st. English Edition 1998:979-981, 5. deutsche Auflage 1998:1000-1003.
2. Kintzel K, Sonntag J, Srauβ E, et al. Neuron-specific Enolase:Reference Values in Cord Blood. Clin Chem Lab Med 1998;36(4):245-247.
3. Butterworth RJ, Wassif WS, Sherwood RA, et al. Serum Neuron-Specific Enolase, Carnosinase and Their Ratio in Acute Stroke. Stroke 1996;27: 2064-2068.
4. Cunningham RT, Watt M, Winder J, et al. Serum neurone-Specific enolase as an indicator of stroke volume. Eur J Clin Invest 1996;26(4):298-303.

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