Elecsys NSE

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REF		Σ	SYSTEM
07299982190* 07299982214*	07299982500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
NSE	10073

Please note

The measured NSE value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the NSE assay method used. NSE values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the NSE assay procedure used while monitoring therapy, then the NSE values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunoassay for the in vitro quantitative determination of neuron-specific enolase (NSE) in human serum. NSE measurements are utilized in monitoring therapy and progress in patients with tumor diseases, particularly small cell lung cancer and neuroblastoma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

NSE is a cell-specific isoenzyme of the glycolytic enzyme enolase. NSE is seen as a valuable tumor marker in monitoring of small cell lung cancer (SCLC), in particular in combination with Pro-gastrin-releasing peptide (ProGRP).[1,2] It has been shown that the levels of NSE in SCLC patients correlate with tumor burden, number of metastatic sites and response to treatment.

Increased levels of NSE have been reported also in non-small cell lung cancer (NSCLC), but the predictive and prognostic role of this marker in NSCLC is still being discussed.[3] Increased serum levels of NSE have been found in all stages of neuroblastoma. The incidence of increased concentration is greater in widespread metastatic disease and correlates with poor prognosis.[3]

Elevated NSE levels may occur in neuroendocrine malignancies but also in a wide variety of other tumor diseases and clinical conditions including melanoma, seminoma, renal cell carcinoma, Merkel cell tumor, carcinoid tumors, dysgerminomas and immature teratomas, malignant pheochromocytoma, cerebral tissue damage due to head injury or following ischaemic stroke, intracerebral hemorrhage, inflammatory brain diseases and Creutzfeldt-Jakob disease.[3]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 µL of sample, a biotinylated monoclonal NSE-specific antibody, and a monoclonal NSE-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as NSE.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-NSE-Ab~biotin, 1 bottle, 18.8 mL:
Biotinylated monoclonal anti-NSE antibody 18E5 (mouse) 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.2; preservative.

R2 Anti-NSE-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 mL:
Monoclonal anti-NSE antibody 84B10 (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 50 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

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Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Do not use plasma.

Centrifuge blood within 1 hour. NSE in erythrocytes and platelets leads to elevated results in hemolyzed or incorrectly centrifuged samples (e.g. extended standing time prior to centrifugation).[4]

Criterion: For concentrations of 0.075-15 ng/mL the deviation is ≤ 2.1 ng/mL. For concentrations > 15 ng/mL the deviation is ≤ 14 %.

Stable for 2 days at 20-25 °C, 5 days at 2-8 °C, 3 months at -20 °C (± 5 °C). Freeze only once.

Note: The stability stated for -20 °C is only valid under the following conditions: deep freeze max. 1 mL sample volume at temperatures lower than -70 °C and then store at -20 °C. When using other freezing procedures, the samples tend to give depressed values.

Ensure that tubes containing separating gel are completely filled.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 12133121122, NSE CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL or
- REF 07360070190, PreciControl Lung Cancer, for 4 x 3.0 mL
- REF 03004864122, Diluent NSE, 4 x 3.0 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Enzymun-Test NSE method.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Lung Cancer or PreciControl Tumor Marker.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations of 0.075-15 ng/mL the deviation is ≤ 1.5 ng/mL. For concentrations > 15 ng/mL the deviation is ≤ 10 %.

Hemolysis interferes because erythrocytes contain NSE.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at NSE concentrations up to 15 µg/mL.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

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In addition, the following special cancer drugs were tested. No interference with the assay was found.

Special cancer drugs

Drug	Concentration tested mg/L
Cyclophosphamide	≤ 150
Cisplatin	≤ 250
Etoposide	≤ 400
Doxorubicin	≤ 50
Vincristine	≤ 2500

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.075-300 ng/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.075 ng/mL. Values above the measuring range are reported as > 300 ng/mL (or up to 600 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.075 ng/mL

Limit of Detection = 0.15 ng/mL

Limit of Quantitation = 0.225 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

An internal study was performed based on guidance from the CLSI protocol EP17-A2. Limit of Blank and Limit of Detection were determined to be the following:

Limit of Blank = 0.0241 ng/mL

Limit of Detection = 0.0741 ng/mL

For Limit of Quantitation ≥ 4 human serum samples were measured over 5 days with 5 replicates per day on one analyzer. With an intermediate precision CV of ≤ 20 %, the Limit of Quantitation was 0.143 ng/mL.

Dilution

Samples with NSE concentrations above the measuring range can be diluted with Diluent NSE. The recommended dilution is 1:2. The concentration of the diluted sample must be > 50 ng/mL.

After manual dilution, multiply the result by the dilution factor.

Expected values

Studies conducted with the Elecsys NSE assay in 3 clinical centers in Germany and by Roche-inhouse covering a total of 547 healthy subjects gave the following results:

16.3 ng/mL (95th percentile)

15.7-17.0 ng/mL (95 % confidence range)

Status: Elecsys NSE Multicenter Evaluation; study No. B99P005, 7/2001.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.411	0.017	4.2	0.028	6.7
Human serum 2	0.471	0.015	3.1	0.029	6.2
Human serum 3	20.4	0.187	0.9	0.275	1.3
Human serum 4	194	2.43	1.3	3.14	1.6
Human serum 5	288	3.41	1.2	4.12	1.4
PC[b)] Tumor Marker1	12.1	0.160	1.3	0.176	1.5
PC Tumor Marker2	95.6	0.931	1.0	1.35	1.4
PC Lung Cancer1	12.8	0.139	1.1	0.182	1.4
PC Lung Cancer2	103	1.25	1.2	1.56	1.5

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys NSE assay, REF 07299982190 (**cobas e** 801 analyzer; y) with the Elecsys NSE assay, REF 12133113122 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 197

Passing/Bablok[5]	Linear regression
y = 0.933x - 0.125	y = 0.966x - 0.874
τ = 0.978	r = 0.999

The sample concentrations were between 0.685 and 279 ng/mL.

b) A comparison of the Elecsys NSE assay, REF 07299982190 (**cobas e** 402 analyzer; y) with the Elecsys NSE assay, REF 07299982190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 158

Passing/Bablok[5]	Linear regression
y = 1.02x - 0.064	y = 1.02x + 0.030
τ = 0.977	r = 0.999

The sample concentrations were between 0.648 and 296 ng/mL.

Analytical specificity

The monoclonal antibodies 18E5 and 84B10 used in the Elecsys NSE assay were raised specifically against the γ-subunit of enolase.

References

1 Molina R, Filella X, Augé JM. ProGRP: A New Biomarker for Small Cell Lung Cancer. EJCMO 2009;1:25-32.

2 Molina R, Marrades RM, Auge JM, et. al. Assessment of a Combined Panel of Six Serum Tumor Markers for Lung Cancer, American Journal of Respiratory and Critical Care Medicine 2016;193(4):428-437.

3 Isgro MA, Bottoni P, Scattena R. Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. Advances in Experimental Medecine and Biology 2015;867:125-43.

4 Schneider J. Tumor markers in detection of lung cancer. Adv Clin Chem 2006;42:1-41.

Elecsys NSE

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5 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has 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 Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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