

REF		$\Sigma$	SYSTEM
12133113122	12133113500	100	cobas e 411 cobas e 601 cobas e 602

## English

## System information

For **cobas e 411** analyzer: test number 770

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 155

## 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 bronchial carcinoma and neuroblastoma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **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).<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>3</sup>

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.<sup>3</sup>

## Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal NSE-specific antibody, and a monoclonal NSE-specific antibody labeled with a ruthenium complex<sup>a)</sup> 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/ProCell 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 reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub><sup>2+</sup>)

## Reagents - working solutions

The reagent rackpack is labeled as NSE.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:  
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-NSE-Ab~biotin (gray cap), 1 bottle, 10 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)<sub>3</sub><sup>2+</sup> (black cap), 1 bottle, 10 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 dust/fume/gas/mist/vapours/spray.
- 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 read in from the respective reagent barcodes.

## Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **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
after opening at 2-8 °C	12 weeks
on the analyzers	8 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).<sup>4</sup>

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.

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, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls 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
- [REF] 07360070190, PreciControl Lung Cancer, for 4 x 3.0 mL
- [REF] 03004864122, Diluent NSE, 4 x 3 mL sample diluent
- General laboratory equipment

## cobas e analyzer

Additional materials for the **cobas e 411** analyzer:

- [REF] 11662988122, ProCell, 6 x 380 mL system buffer
- [REF] 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- [REF] 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

Additional materials for **cobas e 601** and **cobas e 602** analyzers:

- [REF] 04880340190, ProCell M, 2 x 2 L system buffer
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution

- [REF] 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
  - [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
  - [REF] 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
  - [REF] 03023150001, WasteLiner, waste bags
  - [REF] 03027651001, SysClean Adapter M
- Additional materials for all analyzers:
- [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. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

## Calibration

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

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. 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 reagent kit 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 7 days (when using the same reagent kit 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 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.

## Calculation

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

## Limitations - interference

The assay is unaffected by icterus (bilirubin < 1231 µmol/L or < 72 mg/dL), lipemia (triglycerides < 22.8 mmol/L or < 2000 mg/dL) and biotin (< 409 nmol/L or < 100 ng/mL).

**Criterion:** Recovery within ± 10 % of initial value.

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.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at NSE concentrations up to 100000 ng/mL.

In vitro tests were performed on 21 commonly used pharmaceuticals. No interference with the assay was found.

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.

Elevated NSE levels can also occur in the presence of benign lung diseases and malignant neuro-endocrine diseases, such as carcinoid tumors, medullary thyroid carcinoma, Merkel cell tumors of the skin, and carcinoma of the pancreas and adrenal medulla.<sup>5,6,7</sup>

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

0.050-370 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.050 ng/mL. Values above the measuring range are reported as > 370 ng/mL (or up to 740 ng/mL for 2-fold diluted samples).

### Lower limits of measurement

#### Lower detection limit of the test

Lower detection limit: < 0.05 ng/mL

The Lower Detection Limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

### 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.

Multiply the results 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 (95<sup>th</sup> 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 modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	2.58	0.08	3.1	0.11	4.4
Human serum 2	9.32	0.20	2.1	0.36	3.9
Human serum 3	88.0	2.00	2.3	3.87	4.4
PreciControl TM <sup>b)</sup> 1	8.42	0.18	2.1	0.25	3.0

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PreciControl TM2	54.6	1.51	2.8	2.05	3.8
PreciControl LC <sup>c)</sup> 1	12.5	0.224	1.8	0.532	4.3
PreciControl LC2	103	1.43	1.4	4.28	4.1

b) TM = Tumor Marker

c) LC = Lung Cancer

cobas e 601 and cobas e 602 analyzers						
		Repeatability			Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	0.90	0.01	1.6	0.87	0.02	2.2
Human serum 2	11.9	0.09	0.8	11.4	0.35	3.1
Human serum 3	95.1	0.65	0.7	87.3	3.30	3.8
PreciControl TM1	10.2	0.10	1.0	9.87	0.18	1.8
PreciControl TM2	69.8	0.45	0.6	67.3	1.08	1.6

cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PreciControl LC1	11.9	0.200	1.7	0.534	4.5
PreciControl LC2	98.9	0.831	0.8	2.35	2.4

### Method comparison

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

Number of samples measured: 133

Passing/Bablok<sup>8</sup> Linear regression

y = 0.94x + 0.10

y = 0.90x + 1.40

r = 0.907

r = 0.993

The sample concentrations were between 5.8 and 104 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.

### Functional sensitivity

0.25 ng/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of < 20 %.

### References

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- 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.
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- Schneider J. Tumor markers in detection of lung cancer. Adv Clin Chem 2006;42:1-41.
- Sheppard MN, Corrin B, Bennett MH, et al. Immunochemical localization of neuron specific enolase in small cell carcinomas and carcinoid tumours of the lung. Histopathol 1984;8:171-181.

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- 7 Simpson S, Vinik AI, Marangos PJ, et al. Immunohistochemical Localization of Neuron-Specific Enolase in Gastroenteropancreatic Neuroendocrine Tumors. Cancer 1984;54:1364-1369.
- 8 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](http://dialog. Roche.com) for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume for reconstitution
	Global Trade Item Number

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