NSE

cobas®

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170
12133113 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 770
For MODULAR ANALYTICS E170, **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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11, molecular weight approximately 80 kDa) occurs in a variety of dimeric isoforms comprising three immunologically different subunits termed α, β, and γ. The α-subunit of enolase occurs in numerous types of tissue in mammals, whereas the β-subunit is found mainly in the heart and in striated musculature. The enolase isoforms αγ and γγ, which are referred to as neuron-specific enolase (NSE) or γ-enolase, are primarily detectable in high concentrations in neurons and neuro-endocrine cells as well as in tumors originating from them.[1]

Bronchial carcinoma: NSE is described as the marker of first choice in the monitoring of small cell bronchial carcinoma,[1] whereas CYFRA 21-1 is superior to NSE for non-small cell bronchial carcinoma.[2,3,4]

Elevated NSE concentrations are found in 60-81 % of cases of small cell bronchial carcinoma.[1,5]

For NSE there is no correlation to the site of metastasis or to cerebal metastasis,[1,6] but there is good correlation to the clinical stage, i.e. the extent of the disease.[1]

In response to chemotherapy there is a temporary rise in the NSE level 24-72 hours after the first therapy cycle as a result of cytolysis of the tumor cells.[1] This is followed within a week or by the end of the first therapy cycle by a rapid fall in the serum values (which were elevated prior to therapy). By contrast, non-responders to therapy display levels which are constantly elevated or fail to fall into the reference range.[1,7] During remission, 80-96 % of the patients have normal values. Rising NSE values are found in cases of relapse. The rise occurs in some cases with a latent period of 1-4 months, is often exponential (with a doubling time of 10-94 days) and correlates with the survival period.[1] NSE is useful as a single prognostic factor and activity marker during the monitoring of therapy and the course of the disease in small cell bronchial carcinoma: diagnostic sensitivity 93 %, positive predictive value 92 %.[1,5,7]

Neuroblastoma: NSE serum values above 30 ng/mL are found in 62 % of the affected children. The medians rise in accordance with the stage of the disease. There is a significant correlation between the magnitude or frequency of pathological NSE values and the stage of disease; there is an inverse correlation with illness-free survival.[1]

Apudoma: In 34 % of the cases elevated NSE values (> 12.5 ng/mL) are found in serum.[1,8]

Seminoma: 68-73 % of the patients have a clinically significant NSE elevation.[1] There is a utilizable correlation with the clinical course of the disease.

Other tumors: Non-pulmonary malignant diseases show values above 25 ng/mL in 22 % of the cases (carcinomas in all stages). Brain tumors such as glioma, meningioma, neurofibroma, and neurinoma are only occasionally accompanied by elevated serum NSE values. In primary brain tumors or brain metastasis[9] and in malignant melanoma and phaeochromocytoma, elevated NSE values can occur in the CSF (cerebrospinal fluid).[1] Increased NSE concentrations have been reported for 14 % of organ-restricted and 46 % of metastasizing renal carcinomas, with a correlation to the grade as an independent prognosis factor.[1,10]

Benign disease: Elevated serum NSE concentrations (> 12 ng/mL) have been found in patients with benign pulmonary diseases and cerebral diseases. Elevated values, mainly in the liquor, have been found in cerebrovascular meningitis, disseminated encephalitis, spinocerebellar degeneration, cerebral ischemia, cerebral infarction, intracerebral hematoma, subarachnoid hemorrhage, head injuries, inflammatory brain diseases, organic epilepsy, schizophrenia, and Jakob-Creutzfeld disease.[1,11,12]

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[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/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 ($Ru(bpy)_3^{2+}$)

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~$Ru(bpy)_3^{2+}$ (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.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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

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).[1,13]

Stable for 6 hours at 15-25 °C, 24 hours at 2-8 °C, 3 months at -20 °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 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3 mL
- REF 07360070190, PreciControl Lung Cancer, for 4 x 3 mL
- REF 03004864122, Diluent NSE, 4 x 3 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **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

Accessories for MODULAR ANALYTICS E170, **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

Accessories 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 (except for the **cobas e** 602 analyzer).

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). 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.[14,15,16]

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 (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 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 analyzers					
		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

cobas e 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
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[b)]1	8.42	0.18	2.1	0.25	3.0
PreciControl TM2	54.6	1.51	2.8	2.05	3.8
PreciControl LC[c)]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

MODULAR ANALYTICS E170, **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

MODULAR ANALYTICS E170, **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[17]	Linear regression
y = 0.94x + 0.10	y = 0.90x + 1.40
τ = 0.907	r = 0.993

The sample concentrations were between approximately 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

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 Ebert W, Dienemann H, Fateh-Moghadam A, et al. Cytokeration 19 Fragment CYFRA 21-1 Compared with Carcinoembryonic Antigen, Squamous Cell Carcinoma Antigen and Neuron-Specific Enolase in Lung Cancer. Eur J Clin Chem Clin Biochem 1994;32(3):189-199.

3 Vinolas N, Molina R, Galàn MC, et al. Tumor Markers in Response Monitoring and Prognosis of Non-Small Cell Lung Cancer: Preliminary Report. Anticancer Res 1998;18:631-634.

4 Ebert W, Muley TH, Drings P. Does the Assessment of Serum Makers in Patients with Lung Cancer Aid in the Clinical Decision Making Process? Anticancer Res 1996;16(4B):2161-2168.

5 Ebert W, Hoppe M, Muley TH, et al. Monitoring of Therapy in Inoperable Lung Cancer Patients by Measurement of CYFRA 21-1, TPA-TP, CEA and NSE. Anticancer Res 1997(4B);17:2875-2878.

6 Martens P. Serum neuron-specific Enolase as a Prognostic Marker for Irreversible Brain Damage in Comatose Cardiac Arrest Survivors. Acad Emerg Med 1996;3(2):126-131.

7 Fizazi K, Cojean I, Pignon JP, et al. Normal Serum Neuron Specific Enolase (NSE) Value after the First Cycle of Chemotherapy. Cancer 1998;82(6):1049-1055.

8 Kintzel K, Sonntag J, Strauß E, et al. Neuron-Specific Enolase: Reference Values in Cord Blood. Clin Chem Lab Med 1998;36(4):245-247.

9 Jacobi C, Reiber H. Clinical relevance of increased neuron-specific enolase in cerebrospinal fluid. Clin Chim Acta 1988;177(1):49-54.

10 Rasmussen T, Grankvist K, Ljungberg B. Serum gamma-enolase and prognosis of patients with renal cell carcinoma. Cancer 1993;72:1324-1328.

11 Butterworth RJ, Wassif WS, Sherwood RA, et al. Serum Neuron-Specific Enolase, Carnosinase and Their Ratio in Acute Stroke. Stroke 1996;27:2064-2068.

12 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.

13 Wolter W, Luppa P, Breul J, et al. Humorale Tumormarker: Praxis-orientierte Vorschläge für ihren effizienten Einsatz. Dtsch Ärztebl 1996;93(50):C2333-C2339.

14 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.

15 Wick MR, Bernd MD, Scheithauer W, et al. Neuron-specific Enolase in Neuroendocrine Tumors of the Thymus, Bronchia, and Skin. Am J Clin Pathol 1982;79:703-707.

16 Simpson S, Vinik AI, Marangos PJ, et al. Immunohistochemical Localization of Neuron-Specific Enolase in Gastroenteropancreatic Neuroendocrine Tumors. Cancer 1984;54:1364-1369.

17 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, the product information 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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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

Additions, deletions or changes are indicated by a change bar in the margin.

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com