S100

cobas®

S100

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
03175243 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Please note

The measured S100 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 S100 assay method used. S100 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 S100 assay procedure used while monitoring therapy, then the S100 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 S100 (S100 A1B and S100 BB) in human serum. This assay can be used

- to aid in the management of patients suffering from malignant melanoma (Elecsys S100 assay is not suitable for the diagnosis of malignant melanoma)
- to aid in the management of patients after potential brain injury in conjunction with clinical information and imaging techniques

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

Summary

S100 is a small dimeric protein with a molecular weight of approximately 10.5 kD, and belongs to a multigenic family of calcium-binding proteins.[1,2]

S100A1 (α) and S100B (β) were the first members described, originally isolated as an unfractionated mixture by Moore[3] from bovine brain and named S100 after its solubility in a 100 % saturated ammonium sulfate solution. In the meantime, at least 21 different members of the S100 family have been identified.[4]

S100A1 and S100B are predominantly expressed by cells of the central nervous system, mainly astroglial cells, but are also expressed in melanoma cells and to some extent in other tissues. The functional protein, which is composed of hetero- or homodimers of A1 and B, is implicated in a variety of intra- and extracellular regulatory activities.[1,5,6]

In patients suffering from malignant melanoma, especially stage II, III, and IV, elevated S100 serum levels may indicate disease progression. Serial measurements can be useful for follow-up and monitoring therapy success in these patients.[7,8,9,10,11,12,13]

In addition, levels of S100 rise in the CSF (cerebro-spinal fluid) and are released in peripheral blood after a variety of cerebral lesions.

S100 can be detected in patients with cerebral damage caused by several events, e.g. traumatic brain injuries[14,15,16,17,18,19,20,21] or stroke.[22,23,24]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal S100-specific antibody, and a monoclonal S100-specific antibody labeled with a ruthenium complex[a)] react to 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.

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

Reagents - working solutions

The reagent rackpack is labeled as S-100.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-S100-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-S100 antibody (mouse) 1.0 mg/L; phosphate buffer 50 mmol/L, pH 7.2; preservative.

R2 Anti-S100-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-S100 antibody (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

Only the specimen listed below was tested and found acceptable.

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

Do not use plasma.

Stable for 8 hours at 15-25 °C, 2 days at 2-8 °C, 3 months at -20 °C.

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 03289834190, S100 CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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 Combimagazine M, 48 magazines x 84 reaction vessels 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.

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 weighed-out S100 β/β protein.

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 Universal.

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.

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

Calculation

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

Conversion factors:	µg/L x 1 = ng/mL
	µg/L x 1000 = pg/mL
	ng/mL x 1000 = pg/mL

Limitations - interference

The assay is unaffected by icterus (bilirubin < 680 µmol/L or < 40 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

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 1000 IU/mL.

There is no high-dose hook effect at S100 concentrations up to 10 µg/mL.

In vitro tests were performed on 18 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.

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

0.005-39 µg/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.005 µg/L. Values above the measuring range are reported as > 39 µg/L.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 0.005 µg/L

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 S100 concentrations above the measuring range can be diluted with Elecsys S100 Cal1 or S100 negative human serum. Diluent Universal is not recommended. The recommended dilution is 1:5 (manually). The concentration of the diluted sample must be > 1 µg/L.

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

Expected values

- *Apparently healthy adults and patients with malignant melanoma*

Measurements using the Elecsys S100 assay in samples from apparently healthy persons and patients suffering from malignant melanoma in different tumor stages under follow-up investigation revealed the following values:

Group	Subgroup	No. of samples (patients)	Median µg/L	95th percentile	No. of samples above cutoff (> 0.105 µg/L)b)
Apparently healthy adults		206 (206)	0.046	0.105	10 of 206 (4.9 %)
Malignant melanoma patients (all stages under follow-up investigation)	NEDc)	821 (408)	0.044	0.109	45 of 821 (5.5 %)
	Regional lymph node metastases	32 (24)	0.047	0.120	4 of 32 (12.5 %)
	Skin/distant lymph node metastases	21 (15)	0.093	0.511	10 of 21 (47.6 %)
	Distant/visceral metastases	70 (48)	0.077	0.759	30 of 70 (42.9 %)

b) Number of samples > 95th percentile of apparently healthy adults

c) No evidence of disease, tumor free

- *Adult patients with potential brain injury*

Elecsys S100 values were assessed within 3 hours after traumatic event in patients presenting with minor traumatic brain injury (GCS 13-15 - Glasgow Coma Score) and at least one symptom. CCT (cranial computer tomography) was performed within 6 hours after traumatic event. When using the 95th percentile value of the apparently healthy persons (0.105 µg/L) as the cutoff, the following results were obtained for the Elecsys S100 assay compared to the CCT reference:

NPV (negative predictive value) 99.7 %, PPV (positive predictive value) 11 %, sensitivity 98.8 %, and specificity 32.9 % (confidence interval 95 %: NPV 99.1-100 %, PPV 8.8-13.3 %, sensitivity 96.5-100 %, specificity 30-35.9 %).

	CCT positive	CCT negative	Total
Elecsys S100 positve	83	670	753
Elecsys S100 negative	1d)	329	330
Total	84	999	1083

d) 0.098 µg/L

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, human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/L	SD µg/L	CV %	SD µg/L	CV %
Human serum 1	0.084	0.001	1.5	0.004	4.4
Human serum 2	0.203	0.004	2.1	0.011	5.3
Human serum 3	1.93	0.044	2.3	0.107	5.6
PreciControl Ue)1	0.196	0.002	1.0	0.006	3.1
PreciControl U2	2.65	0.019	0.7	0.084	3.2

e) U = Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability			**Intermediate precision**	
Sample	Mean µg/L	SD µg/L	CV %	SD µg/L	CV %
Human serum 1	0.089	0.001	1.6	0.002	2.3
Human serum 2	0.216	0.003	1.6	0.006	2.7
Human serum 3	2.05	0.029	1.4	0.062	3.0
PreciControl U1	0.199	0.002	0.9	0.007	3.3
PreciControl U2	2.61	0.012	0.5	0.094	3.6

Method comparison

A comparison of the Elecsys S100 assay (y) with Liamat Sangtec100 (x_1) and Liaison Sangtec100 (x_2) using clinical samples from patients with malignant melanoma gave the following correlations:

Passing/Bablok[25]

Elecsys/Liamat (x_1)

$y = 0.550x_1 + 0.025$

$r = 0.729$

Number of samples measured: 934

The sample concentrations were between approximately 0.00 and 9.87 µg/L.

Elecsys/Liaison (x_2)

$y = 0.783x_2 + 0.003$

$r = 0.857$

Number of samples measured: 379

The sample concentrations were between approximately 0.01 and 2.08 µg/L.

Analytical specificity

Cross reactivity against S100A1 (αα) dimers has been found < 1 %.

Functional sensitivity

< 0.02 µg/L

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

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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

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