DiaSorin Italia S.p.A.
Via Crescentino snc - 13040 Saluggia (VC) - Italy
www.diasorin.com

Changes: Update of Legal Manufacturer name
Deletions: -

LIAISON® S100 (REF 314701)

1. INTENDED USE

LIAISON® S100 is a quantitative automated chemiluminescent immunoassays (CLIA) for the *in vitro* determination of protein S-100B in human serum and CSF (Cerebrospinal fluid). LIAISON® S100 is intended for use as an aid in the management of patients suffering from malignant melanoma. The test has to be performed on the LIAISON® Analyzer family.

2. SUMMARY AND EXPLANATION OF THE TEST

Protein S-100B (S-100) belongs to the S-100-calmodulin-troponin C-superfamily of EF-hand calcium binding proteins. The S-100 proteins constitute a multigenic family of low molecular weight (9-13 kDa) Ca^{2+}-binding proteins. The first member of the S-100 family was originally isolated by Moore (1965) from bovine brain tissue and named S-100, due to its solubility in 100% saturated ammonium sulphate at neutral pH. The biological properties of the different forms of S-100 are intimately related to the Ca^{2+} metabolism, due to the Ca-binding properties of S-100. Calcium binding proteins are involved in many different activities like cell growth and differentiation, energy metabolism, cell structure and intracellular signal transduction.
So far 20 different S-100 proteins have been identified based on structural and functional similarities. The S-100B protein exists in two different dimeric forms S-100A1B and S-100BB made up of S-100A1 (93 residues) and S-100B (91 residues) subunits.
S-100 proteins show a specific pattern of tissue and cellular distribution. S-100B is most abundant in glial and Schwann cells of the central and peripheral nervous system and is also well expressed in melanocytes, adipocytes and chondrocytes. S-100A1 is found in astrocytes, neurons, striated muscles, heart and kidney.
Malignant melanoma is a malignant neoplasm of the melanocytes. Due to the high concentration of S-100B in malignant melanocytes, S-100B was evaluated as marker for malignant melanoma. In a large number of publications it has been demonstrated that S-100B is a reliable tumour marker mainly in metastatic melanoma where it reflects the tumour load and prognosis. The number of melanoma patients showing positive S-100B values increases with tumour stage and increased levels of S-100B are found in about 68-92% of patients with stage IV disease.
S-100B has also been described to be a valuable marker for monitoring and follow-up of melanoma patients. Monitoring the progression of the disease is critical in the management of the disease. S-100B follows the course of the disease and has been shown to be an early indicator of relapse. It could therefore be a valuable adjunct to imaging and clinical assessment.
Another important clinical application for S-100B is as a marker for different forms of cerebral lesions. Clinical studies have demonstrated an immediate increase of S-100B in the circulation after a brain injury indicating a fast release of brain S-100B into the blood. The release mechanism of protein S-100B is however not yet fully understood.
S-100B is a very sensitive marker for an injury of the brain caused by head trauma and is released at the time of the primary injury. The level in serum strongly correlates with the severity of the primary trauma and the outcome of the patient. In patients with global cerebral ischemia, it has been reported that a positive value in the serum 24 hours after ischemia is an independent predictor of not regaining consciousness.
S-100B has also been shown to be a useful marker in stroke and birth asphyxia.

3. PRINCIPLE OF THE PROCEDURE

LIAISON® S100 is a two-site chemiluminescent immunoassay (CLIA) (sandwich principle) based on paramagnetic particles coated with two monoclonal antibodies and a monoclonal conjugate antibody labelled with an isoluminol derivative. During a first incubation, S-100 present in calibrators, samples or controls binds to the solid phase monoclonal antibodies, and subsequently after a washing step in a second incubation the antibody conjugate reacts with S-100 already bound to the solid phase.
After incubation, the unbound material is removed with a wash cycle.
Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of S-100 concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

The order of reagents reflects the layout of containers in the reagent integral.

Reagent Integral for 100 determinations			
2.3	mL	SORB	Solid Phase: containing magnetic particles coated with monoclonal antibodies (mouse), BSA, 0.09% sodium azide.
4.0	mL	DIL SPE	Specimen Diluent (for CSF only): containing 0.09% sodium azide.
23.0	mL	CONJ	Conjugate: containing monoclonal antibody (mouse) labelled with isoluminol, BSA, 0.09% sodium azide.
14.0	mL	BUF A	Buffer A: containing mouse IgG, BSA, 0.09% sodium azide.

All reagents are provided ready-to-use.

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® Analyzer**
LIAISON® Wash/System Liquid (REF 319100)	LIAISON® Wash/System Liquid (REF 319100)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)
LIAISON® XL Starter Kit (REF 319200)	LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200)
LIAISON® XL Disposable Tips (REF X0015)	LIAISON® Cleaning Kit (REF 310990)
	LIAISON® Light Check 12 (REF 319150)

Additionally required materials

LIAISON® Control S100 (REF 319112)

LIAISON® S100 Cal (low/high) (REF 319117) **ONLY DELIVERED TOGETHER WITH** LIAISON® S100 (REF 314701)

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.
As, however, no absolute assurance can be given that pathogens are absent, all components of human and animal origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics during the assay.
Do not pipette by mouth.
Avoid direct contact with all potentially infectious materials by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.
Avoid splashing or forming an aerosol. Any drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.
All samples and reagents containing biological materials used for the assay must be considered potentially able to transmit infectious agents; the waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Reagents containing sodium azide (< 0.1%) [EC no: 247-852-1]:

DIRECTIVE	**EC No. 1272/2008**
HAZARD / RISK STATEMENTS	**EUH 210 -** Safety data sheet available on request

7. REAGENT PREPARATION

7.1. Reagent Integral

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:
Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Repeat as necessary until the magnetic particles are completely resuspended. After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:
Visually inspect the reagents, calibrators in particular to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

Place the integral into the reagent area of the analyzer with the barcode label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL Analyzer

LIAISON® XL Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
Insert the reagent integral into the dedicated slot.
Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
Follow the analyzer operator's manual to load the specimens and start the run.

7.2. Controls
Refer to the LIAISON® Control S100 Set instructions for use section for proper preparation and handling instructions.

7.3. Calibrators
Refer to the LIAISON® S100 Cal (low/high) Set instruction for use for proper preparation and handling instructions.
NOTICE: The LIAISON® S100 Kit (REF 314701) is only to be used in conjunction with the *bundled* LIAISON® S100 Cal (low/high) (REF 319117). Do not use other LIAISON® S100 Cal (low/high) lots!

8. REAGENT STORAGE AND STABILITY

8.1. Reagent integral
Sealed: Stable at 2-8 °C until the expiry date.
Opened onboard or at 2-8 °C: Stability 2 weeks (see § 12).
After this period, it is still possible to keep on using the reagent integral provided that the controls are found within the expected ranges.
Use always the same LIAISON® Analyzer for a reagent integral already opened.
Do not freeze.
Keep upright for storage to facilitate later proper resuspension of magnetic particles.
Use storage rack provided with the LIAISON® Analyzer family for upright storage of reagent integral.
Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

Serum and CSF can be used.
If the test is not performed on the day of sample collection, the serum should be separated from the sediment and be stored in a separate tube.
Storage at 2-8 °C: 24 h.
For longer storage periods: freeze below -20 °C.
Avoid repeated freezing and thawing cycles.
Stored samples should be thoroughly mixed prior to use (Vortex mixer).
Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested.
Do not use clotted samples.
The minimum volume required for a single determination is 250 µL specimen (100 µL specimen + 150 µL dead volume).

10. CALIBRATION

Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Up to 4 calibrations can be performed (in total).
Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

- A new lot of reagent integral or of Starter Kit is used.
- The previous calibration was performed more than 14 days before.
- The analyzer has been serviced.
- Control values lie outside the expected ranges.

LIAISON® Analyzer: Calibrator values are stored in the reagent integral label.
LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency Identification Transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.
LIAISON® Analyzer: Each test parameter is identified via barcodes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.
LIAISON® XL Analyzer: Each test parameter is identified via information encoded in the reagent integral Radio Frequency Identification Transponder (RFID Tag). In the event the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction

The LIAISON® Analyzer operations are as follows:

1. Dispense Buffer A into the reaction Module
2. Dispense sample, calibrator or controls
3. Dispense coated magnetic particles (Solid Phase)
4. Incubate
5. Wash with Wash/System Liquid
6. Dispense Conjugate into the reaction Module
7. Incubate
8. Wash with Wash/System Liquid
9. Add the Starter reagent and measure the light emitted

The LIAISON® XL Analyzer operations are as follows:

1. Dispense sample, calibrator or controls into the reaction Cuvette
2. Dispense coated magnetic particles (Solid Phase) and Buffer A
3. Incubate
4. Wash with Wash/System Liquid
5. Dispense Conjugate into the reaction Cuvette
6. Incubate
7. Wash with Wash/System Liquid
8. Add the Starter reagent and measure the light emitted

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control could be performed by running the LIAISON® control sera or dedicated commercial controls

- at least once per day of use,
- whenever a new reagent integral is used,
- whenever the kit is calibrated,
- whenever a new lot of Starter Reagents is used,
- to assess adequacy of performance of the open integral beyond 2 weeks,
- or in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lay within the expected ranges: whenever one of the controls lies outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.
The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates S-100 concentrations for the unknown samples in µg/L. For details, refer to the analyzer operator's manual.
Calibrators and controls may give different RLU or dose results on LIAISON® and LIAISON® XL, but patient results are equivalent.

13.1. Assay range

The LIAISON® S100 measures concentrations up to 30 µg/L.

13.2. Reference range

95% of healthy men and women were found to have S-100 values below 0.15 µg/L in serum and below 2.7 µg/L in CSF.
Each laboratory should establish its own reference range.

14. LIMITATIONS OF THE PROCEDURE

The reagents should be used only in the LIAISON® Analyzer family.
Single components of the reagent integral should not be removed from the integral.
This kit must not be used after the expiry date printed on the package label.
A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
Bacterial contamination or heat inactivation of the specimens may affect the test results.
Patients may exhibit S-100 values within the reference range. S-100 levels may only be interpreted in context with the clinical picture and other diagnostic procedures.
Any therapeutical decision must also be taken on a case-by-case basis.
Although HAMA-neutralizing agents are added, extremely high HAMA (human anti-mouse antibodies) concentrations may occasionally influence results.
Liquor samples (CSF) containing S-100 levels above the measuring range may be prediluted with diluent.
Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON® or LIAISON® XL).

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g, hemolysis, lipemia, bilirubinemia).

15.2. Interference

Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by concentrations of bilirubin < 0.2 mg/mL, hemoglobin < 125 mg/dL or triglycerides < 30 mg/mL.

15.3. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of S-100, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability).

Intra-assay variation			Inter-assay variation		
Mean value (µg/L)	CV (%)	n*	Mean value (µg/L)	CV (%)	n*
0.11	6.4	5	0.13	8.5	21
0.31	3.1	5	0.27	9.3	21
1.60	2.8	5	1.82	5.7	25
2.60	3.9	5	2.95	7.6	26
9.90	2.8	5	7.67	6.7	25
18.40	3.6	5	18.40	5.6	27

* number of determinations

15.4. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of S-100, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability).

Intra-assay variation			Inter-assay variation		
Mean value (µg/L)	CV (%)	n*	Mean value (µg/L)	CV (%)	n*
0.14	3.2	20	0.12	4.3	20
0.33	2.9	20	0.82	2.5	20
1.71	2.6	20	3.53	3.4	20
5.59	2.5	20	13.29	4.2	20

* number of determinations

15.5. Trueness

The assay trueness has been checked by the dilution and recovery tests.

15.6. Dilution test

Liquor samples (CSF) containing high S-100 concentrations were tested as such and after serially diluting with the specimen diluent. S-100 concentrations measured versus expected were analyzed by linear regression. LIAISON® S100 Diluent can only be used for the dilution of high S-100 CSF samples. Serum samples should be diluted in low titred S-100 serum (not provided in kit).

The table gives an example of serum diluted with low titred S-100 serum. Original concentration: 10.7 µg/L.

Dilution	Measured value (µg/L)	Expected value (µg/L)	Recovery (%)
1 : 2	5.94	5.39	110
1 : 4	2.98	2.74	109
1 : 8	1.48	1.41	105
1 : 16	0.85	0.75	113
1 : 32	0.46	0.42	109

The table gives an example of CSF diluted with the diluent. Original concentration: 2.176 µg/L.

Dilution	Measured value (µg/L)	Expected value (µg/L)	Recovery (%)
1 : 2	1.21	1.09	111
1 : 4	0.60	0.54	111
1 : 8	0.28	0.27	104
1 : 16	0.14	0.14	100
1 : 32	0.07	0.07	100

15.7. Recovery test

Samples spiked with S-100 were tested to evaluate the recovery of the LIAISON® S100 assay.
The table gives an example of the recovery in a patient sample (0.14 µg/L) spiked with different amounts of S-100.

Measured value (µg/L)	Expected value (µg/L)	Recovery (%)
0.80	0.83	97
1.63	1.74	93
3.34	3.35	100
9.48	10.38	91

15.8. High-Dose Hook Effect

There is no high-dose hook effect for S-100 concentrations up to 2400 µg/L.
Whenever samples containing extremely high analyte concentrations are tested, the HDH can mimic concentrations lower than real. Analysis of HDH was evaluated by testing five high-concentration S-100-spiked samples. All samples resulted in calculated concentration values above the measuring range, indicating no sample misclassification.

15.9. Analytical sensitivity

Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 2 standard deviations.

	Analytical sensitivity
LIAISON® Analyzer family	0.02 µg/L

16. REFERENCES

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Changes: Update of Legal Manufacturer name
Deletions: -

1. INTENDED USE

LIAISON® S100 Cal (low/high) is used for the calibration of the LIAISON® S100 assay. Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration. Calibrators are required for the LIAISON® S100 to be performed on the LIAISON® Analyzer family.

2. MATERIAL PROVIDED

Calibrator reagent for 2 determinations in triplicate per vial:					
2	x	1.0	mL	CAL 1	Calibrator low: containing S-100B antigen from bovine brain, Ovalbumin (chicken), 0.09% sodium azide
2	x	1.0	mL	CAL 2	Calibrator high: containing S-100B antigen from bovine brain, Ovalbumin (chicken), 0.09% sodium azide
8	x	white		CAL 1 Barcode label, small	For labeling the aliquoted calibrator tubes
8	x	white		CAL 2 Barcode label, small	For labeling the aliquoted calibrator tubes

The reagents are lyophilized.
Reconstituted reagents are slightly turbid.

NOTICE: The LIAISON® S100 Cal (low/high) (REF 319117) is only to be used in conjunction with the *bundled* LIAISON® S100 Kit (REF 314701). Do not use other LIAISON® S100 Kit (REF 314701) lots!
For additional details such as proper test performance of LIAISON® S100 consult the instructions for use for LIAISON® S100.

The calibrators consist of S-100 protein. This S-100 protein originates from purified and well-characterized material. Values of calibrators are assigned against in-house reference S-100 material.
LIAISON® Analyzer: Calibrator values are stored in the reagent integral label.
LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency Identification Transponder (RFID Tag).

3. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.
As, however, no absolute assurance can be given that pathogens are absent, all components of human and animal origin should be considered potentially infectious and handled with care.

4. SAFETY PRECAUTIONS

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Do not pipette by mouth.
Avoid direct contact with all potentially infectious materials by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.
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All samples and reagents containing biological materials used for the assay must be considered potentially able to transmit infectious agents; the waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Reagents containing sodium azide (< 0.1%) [EC no: 247-852-1]:

DIRECTIVE	**EC No. 1272/2008**
HAZARD / RISK STATEMENTS	**EUH 210 -** Safety data sheet available on request

5. PREPARATION OF THE REAGENT

Reconstitution
Reconstitute with 1.0 mL deionized or distilled water.
Allow the vials to stand for 15 minutes, at approximately 18-25 °C.
Mix thoroughly by gentle inversion, avoid foaming.
If necessary, aliquot the calibrators as directed in paragraph 6.
For details on the use of the calibrators, refer to the analyzer operator's manual.
Refer to paragraph 6 for details on the storage of the calibrators.

6. STORAGE AND STABILITY

Upon receipt, the calibrators must be stored at 2-8 °C in an upright position to prevent adherence to the vial cap.
Lyophilized: At 2-8 °C until the expiry date.
Reconstituted: 2 hours onboard. The calibrators should be used within this period.
Frozen: Aliquots can be stored at -20 °C for up to 5 weeks.
Immediately after complete reconstitution it is possible to aliquot the calibrators by pipetting 480 µL into each of two tubes that have been labeled using the provided white labels and deep-freeze them. After thawing the calibrators must be used within 2 hours.
During handling, use appropriate precautions to avoid bacterial contamination of calibrators.

7. HANDLING

Use the patient rack type “L” when using supplied glass vials. When the calibrators should be aliquoted please use appropriate patient rack type “A”. The calibrator data is read by the barcode reader and displayed in the dialog box of editing samples.
For proper handling please refer to the analyzer operator´s manual.