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LDN®

Instructions for use

S 100B ELISA

REF **TM E-4800**

Σ 96

+2 +8 °C

IVD

CE

S100B ELISA

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of S100B concentration in human serum and plasma.

S100B ELISA kit is intended for laboratory use only.

CLINICAL SIGNIFICANCE

S100 is a 20 kDa protein belonging to the S100/calmodulin/troponin C superfamily of EF-hand calcium-binding proteins. S100 was originally isolated from human brain and considered a glial-cell specific protein (1). Today, 20 monomers of the S100 family have been identified based on structural and functional similarities (2,3). Most of the S100 proteins exist as dimers and are expressed in a cell-specific manner. Two of the S100 monomers, designated S100α and S100β (4) are highly conserved between species and are found as homo- (ββ) and heterodimers (αβ) in central nervous system glial cells and in certain peripheral cells eg. Schwann cells, melanocytes, adipocytes, and chondrocytes (5). S100αβ and S100ββ are also present in malignant tissues, most notably in melanoma and to a lesser extent in glioma, thyroid cell carcinoma and renal cell carcinoma (2).

Determination of S100B (like S100αβ and S100ββ units) in serum has been shown to be clinically useful for prognosis and treatment monitoring of patients diagnosed with malignant melanoma (6-9). Studies also suggest that S100B may be useful in the management of patients with brain damage from eg. traumatic head injury, perinatal asphyxia, cardiac arrest, cardiac surgery and stroke (10-13).

PRINCIPLE

The S100B ELISA TEST is based on binding of S100B by two antibodies, one immobilized on microwell plates, and the other one conjugates with horseradish peroxidase (HRP).

The assay is a two steps binding procedure and after every incubation step, the bound/free separation is performed by a simple solid-phase washing.

Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H_2SO_4) is added..
The color intensity is proportional to the S100B concentration in the sample. S100B concentration in the sample is calculated through a calibration curve.

REAGENTS, MATERIALS AND INSTRUMENTATION

Reagents and materials supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**TM E-4801**	Standard 0	0 pg/mL	1 ml
STANDARD B	**TM E-4802**	Standard 1	80 pg/mL	1 ml
STANDARD C	**TM E-4803**	Standard 2	160 pg/mL	1 ml
STANDARD D	**TM E-4804**	Standard 3	320 pg/mL	1 ml
STANDARD E	**TM E-4805**	Standard 4	2000 pg/mL	1 ml
STANDARD F	**TM E-4806**	Standard 5	4500 pg/mL	1 ml

6x (1 vial = lyophilized); STD0 - STD5

CONTROL 1 **+** CONTROL 2 **TM E-4851 + TM E-4852 Controls**

2x (1 vial = lyophilized); Negative Control; Positive Control

CONJUGATE-BUFF **TM E-4839 Conjugate buffer**

(1 bottle) 20 mL; Tris buffer; BSA 10 g/L, Tween 0.05%

CONJUGATE-CONC **TM E-4840 Conjugate**

(1 vial) 1 mL; Anti-S100B-HRP conjugate

ASSAY-BUFF **TM E-4813 Assay buffer**

(1 bottle) 12 mL; Tris buffer; BSA 10 gr/L, stabilizing reagent

96 **TM E-4831 Coated microplate**

(1 microplate breakable); Anti-S100B adsorbed on microplate

SUBSTRATE **MS E-0055 TMB-Substrate**

(1 bottle) 15 mL; $_2O_2$-TMB 0.25 g/L (avoid any skin contact)

STOP-SOLN **MS E-0080 Stop solution**

(1 bottle) 15 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

WASH-CONC 50x **SA E-0030 Conc.wash solution 50x**

(1 bottle) 20 mL; NaCl 45g/L Tween 20 55g/L

Necessary reagents not supplied with the kit

Distilled water.

Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader(450 nm)

Notes

Store all reagents between 2 °C - 8 °C in the dark.

Open the bag of reagent 6 (Coated Microplate) only when it is at room temperature and close it immediately after use.

Do not remove the adhesive sheets on the strips unutilized

WARNINGS

This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.

Use appropriate personal protective equipment while working with the reagents provided.

Follow Good Laboratory Practice (GLP) for handling blood products.

Some reagents contain small amounts of Sodium Merthiolate or Proclin 300 as preservative. Avoid the contact with skin or mucosa.

The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.

The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.

Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

This method allows the determination of S100B from 10 to 5000 pg/mL.

PRECAUTIONS

Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.

All reagents should be stored refrigerated at 2 °C - 8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.

Allow all kit components and specimens to reach room temperature (22 °C - 28 °C) and mix well prior to use.

Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.

If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.

The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.

It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate

Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

Maximum precision is required for reconstitution and dispensation of reagents.

Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.

Plate readers measure vertically. Do not touch the bottom of the wells.

PROCEDURE

Preparation of the sample

The S100B determination can be carried out in human serum or plasma. Do not use hemolyzed samples.
Samples can be stored at 2 °C - 8 °C for 1 day; for long periods store at -20 °C.
Avoid repetitive freezing and thawing of samples.
Do not leave the samples at room temperature (22 °C - 28 °C) for long period.
For sample with concentration higher than 5 ng/mL dilute the sample with Assay buffer.

Preparation of the Standards and Controls

Reconstitute standards and controls with 1 mL of distilled water before use.
Once reconstituted they are stable for 4 weeks at 2 °C - 8 °C and about six month if stored at -20 °C.
It is advised to divide the reconstituted content in small aliquots and store them at -20 °C.
Avoid repetitive freezing and thawing and long time exposure at room temperature (22 °C - 28 °C).
The values of Standard concentration are lot specific and are reported on the vial labels.

Preparation of the Conjugate

Prepare 2 hours before use.
Add 50 µL Conjugate (reagent 4) to 1.0 mL of Conjugate Buffer (reagent 3).
The quantity of diluted conjugate is proportional to the number of tests.
Mix gently for 5 minutes, with rotating mixer.
Stable for 3 hours at room temperature (22 °C - 28 °C).

Preparation of the wash solution

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use.
For smaller volumes respect the 1:50 dilution ratio.
The diluted wash solution is stable for 30 days at 2 °C - 8 °C.

Procedure

Allow all reagents to reach room temperature (22 °C - 28 °C) for at least 30 minutes.

Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2 °C - 8 °C.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the standard curve (S0-S5), two for each Control, two for each sample, one for Blank.

Pipette:

Reagent	Standard	Sample / Control	Blank
Standard S0-S5, Controls	50 µL		
Sample / Control		50 µL	
Assay Buffer	50 µL	50 µL	
Incubate 2 hours at room temperature (22 °C – 28 °C). Remove the contents from each well, wash the wells 6 times with 300 µL of diluted wash solution			
Diluted Conjugate	100 µL	100 µL	
Incubate 1 h at room temperature (22 °C – 28 °C). Remove the content from each well, wash the wells 6 times with 300 µL of diluted wash solution			
TMB-Substrate	100 µL	100 µL	100 µL
Incubate 30 minutes at room temperature (22 °C – 28 °C), in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

RESULTS

Mean Absorbance

Calculate the mean of the absorbencies (Em) for each point of the standard curve and of each sample.

Standard Curve

Plot the values of absorbance of the standards (S_0 –S_5) against concentration. Draw the best-fit curve through the plotted points (e.g.: Cubic spline or Four Parameter Logistic).

Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations in pg/mL.

REFERENCES VALUES

Each laboratory must establish its own normal ranges based on patient population:

Normal range: Mean = 50 pg/mL; SD = 15 pg/mL

Pathological limit: > 75 pg/mL

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

PERFORMANCE CHARACTERISTICS

Specificity

The antibody recognizes specifically the β subunit therefore reacts with S100αβ and S100ββ units.

It is not reactive against the S100αα unit.

Cross-reacts with S100 from bovine, porcine, rabbit, cat and rat.

Does not react with the other EF-hand family proteins.

Sensitivity

The lowest detectable concentration of S100B that can be distinguished from the Calibrator 0 is 35,27 pg/mL.

Hook Effect

In this assay, no hook effect is observed up to 5000 pg/mL of S100B

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations

TROUBLESHOOTING

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

no conjugate pipetted
contamination of conjugates and/or of substrate
errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

incorrect conjugate (e.g. not from original kit)
incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

incorrect conjugate (e.g. not from original kit)
incubation time too long, incubation temperature too high
water quality for wash buffer insufficient (low grade of deionization)
insufficient washing (conjugates not properly removed)

Unexplainable outliers

contamination of pipettes, tips or containers -insufficient washing (conjugates not properly removed)

too high within-run CV%

reagents and/or strips not pre-warmed to Room Temperature prior to use
plate washer is not washing correctly (suggestion: clean washer head)

too high between-run CV %

incubation conditions not constant (time, temperature)
controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
person-related variation

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!