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DCM074-4
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S100B ELISA

for routine analysis

Direct immunoenzymatic determination of S100B in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO074

INTENDED USE

Eagle Biosciences S100B ELISA Assay Kit is an immunoenzymatic colorimetric method for quantitative determination of S100B concentration in human serum or plasma. S100B ELISA Assay Kit is intended for research use only.

1. 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).

2. PRINCIPLE

S100B ELISA Assay Kit is based on binding of S100B by two antibodies, one immobilized on microwell plates, and the other one conjugated 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.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit.

1. S100B Calibrators (6 vials, lyophilized)

CAL0	REF DCE002/7406-0
CAL1	REF DCE002/7407-0
CAL2	REF DCE002/7408-0
CAL3	REF DCE002/7409-0
CAL4	REF DCE002/7410-0
CAL5	REF DCE002/7411-0

2. Controls (2 vials, lyophilized)

Negative Control	REF DCE045/7401-0
Positive Control	REF DCE045/7402-0

3. Conjugate buffer (1 vial, 20 mL)
Tris buffer; BSA 10 g/L, tween 0.05%
REF DCE044-0

4. Conjugate (1 vial, 1 mL)
Anti S100B antibody conjugated with horseradish peroxydase (HRP) REF DCE002/7402-0

5. Assay buffer (1 vial, 12 mL)
Tris buffer; BSA 10 g/L REF DCE043-0

6. Coated Microplate (1 breakable microplate)
Anti S100B antibody adsorbed on microplate
REF DCE002/7403-0

7. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

8. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005-0

9. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45g/L Tween20 55g/L REF DCE006-0

3.2 Necessary reagents not supplied with the kit

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Notes

Store all reagents between 2-8°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.

4. WARNINGS

- This S100B ELISA Assay Kit is intended for in research 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 of the S100B ELISA Assay Kit contain small amounts of Sodium Merthiolate or Proclin 300^{R} 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.

5. 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 of the S100B ELISA Assay Kit should be stored refrigerated at 2-8°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 S100B ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange S100B ELISA Assay 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.

6. PROCEDURE

6.1 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 2-8°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 (22-28°C) for long period.

For sample with concentration over 5 ng/mL dilute the sample with Assay buffer

6.2 Preparation of the Calibrators and Controls

Reconstitute the Calibrators and Controls with 1 mL of distilled water before use.

Once reconstituted they are stable for 4 weeks at 2-8°C and about six months if stored at -20°C. It is advised to aliquot the reconstituted content in small aliquots and store them at -20°C. Avoid repetitive freezing and thawing and long time exposure at room temperature (22-28°C).

The value of Calibrators concentration is lot specific and is reported on the vial labels.

6.3 Preparation of the Conjugate

Prepare 2 hours before use.

Add 50 µL of Conjugate (reagent 4) to 1 mL of Conjugate Buffer (reagent 3). The quantity of diluted conjugate is proportional at the number of the tests.

Mix gently for 5 minutes, with a rotating mixer.

Stable for 3 hours at room temperature (22-28°C).

6.4 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÷8°C.

6.5 Procedure

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

- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°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 calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_5	**50 µL**		
Sample/ Controls		**50 µL**	
Assay Buffer	**50 µL**	**50 µL**	
Incubate 2 h at room temperature (22–28°C). Remove the content 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 (22–28°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÷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.			

7. RESULTS

7.1 Mean Absorbance
Calculate the mean of the absorbencies (Em) for each point of the calibration curve (C_0-C_5) and of each sample.

7.2 Calibration curve
Plot the values of absorbance (Em) of the Calibrators (C_0-C_5) against concentration. Draw the best-fit curve through the plotted points (ex: Cubic spline or Four Parameter Logistic).

7.3 Calculation of Results
Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations in pg/mL.

8. REFERENCES VALUES
Each laboratory must establish its own normal ranges based on sample 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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

9. PERFORMANCE CHARACTERISTICS

9.1 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 reacts with the other EF-hand family proteins.

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

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

10. WASTE MANAGEMENT
Reagents must be disposed off in accordance with local

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DiaMetra S.r.l. Headquater: Via Garibaldi, 18
20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039–02–2133354.
Manufactory: Via Pozzuolo 14, 06038 SPELLO (PG) Italy
Tel. 0039-0742–24851
Fax 0039–0742–316197
E-mail: info@diametra.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

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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

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

too high between-run

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