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1   INTENDED USE

The Human S100B ELISA is a HRP labelled antibody based sandwich enzyme immunoassay for the quantitative measurement of human S100B protein in serum, cerebrospinal fluid, heparin plasma and tissue culture medium.   It is intended for *in vitro* and *research use* only.

Features

− The total assay time is less than four hours.
− The kit measures total serum, liquor or heparin plasma S100B.
− Quality controls are human serum based. Animal serum is used for Dilution Buffer preparation.
− The components are ready-to-use (with the exception of calibrators, quality controls and wash solution).

2   STORAGE, EXPIRATION

Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

3   SUMMARY

S-100B is a member of highly homologous Ca^{2+} binding proteins family that possess two EF-hand motifs. The family of S100 proteins consists of 19 members. Most S100 proteins exist as dimers (frequently homodimers) within cells. Exclusively expressed in vertebrates, S100 is implicated in various intracellular and extracellular regulatory activities. Studies indicate that S100 proteins are involved in the inhibition of protein phosphorylation, inhibition of cytosceletal constituent assembly, regulation of Ca^{2+} homeostasis, stimulation of enzyme activities, and interaction with transcription factors. S100B is abundant in the nervous system where it is predominantly expressed in astrocytes, oligodendrocytes and Schwann cells. When secreted by astrocytes, S100B has neurotrophic effects during development and nerve regeneration at physiologic (nanomolar) concentrations. However high (micromolar) concentrations of S100B have shown to be neurotoxic, participating in the physiology of neurodegenerative disorders. The clinical values have been demonstrated in stroke, cerebral complications association with cardiac arrest and in patients with severe as well as minor head injury. Patients with progressive melanoma disease also show elevated serum concentrations of S100B.

4   TEST PRINCIPLE

In the Human S100B ELISA, calibrators, quality controls and samples are incubated with polyclonal anti-cow S100B antibody coated in microtitration wells. After 90 minutes incubation and washing, monoclonal anti-human S100B antibody labelled with horseradish peroxidase (HRP) is added to the wells and incubated with captured S100B. After 90 minutes incubation and another washing step, the remaining conjugate is allowed to react with the substrate tetramethylbenzidine and H_2O_2. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow colour product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of S100B.

A standard curve is constructed by plotting absorbance values versus S100B concentrations of calibrators and concentrations of unknown samples are determined using this standard curve.

5   PRECAUTIONS

− For in vitro use and research use only.

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– This kit contains components of human and animal origin. These materials were found not to be reactive for hepatitis B surface antigen and for HIV antibody. However, these materials should be handled as potentially infectious, as no tests can guarantee the complete absence of infectious agents.

– Avoid contact with the acidic Stop Solution and Substrate Solution that contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.

– Wear gloves and laboratory coats when handling immunodiagnostic materials.

– The materials must not be pipetted by mouth.

– Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

– Reagents with different lot numbers should not be mixed.

– Reagents should not be used beyond the expiration marked on kit label.

6   REAGENTS SUPPLIED

1. Antibody Coated **Microtiter Strips** (96 wells), coated with polyclonal Anti-Cow S100B Antibody, vacuum sealed.

2. **Conjugate Solution** (Horseradish Peroxidase labelled Anti-Human S100B Antibody), 13 mL

3. S100B Master **Standard**, lyophilized, 1 vial.

4. **Control High**, lyophilized, 1 vial.

5. **Control Low**, lyophilized, 1 vial.

6. **Dilution Buffer**, ready to use, 20 mL

7. **Wash Solution Concentrate** (10x concentrated), 100 mL

8. **Substrate Solution** (TMB), ready to use, 13 mL

9. **Stop Solution** (0.2 M H_2SO_4), ready to use, 13 mL

10. Instruction Manual + Certificate of Analysis

7   REAGENTS REQUIRED BUT NOT SUPPLIED

– Test tubes for diluting samples

– Precision pipettes to deliver 50-1000 μL and disposable tips

– Multichannel pipette 100 μL

– Microplate reader with 450 ± 10 nm filter

– Software package facilitating data generation and analysis (optional)

– Orbital microplate shaker capable of approximately 300 rpm (optional)

– Microtitration plate washer (optional) [Manual washing is possible but not preferable.]

– Absorbent material for blotting the microtiter plate

– Glassware (graduated cylinder and bottle for Wash Solution)

– Deionized (distilled) water

8   PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to the assay.

If you do not use the whole plate, return unused strips in the provided aluminium bag with desiccant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Assay reagents are supplied ready-to-use, with the exception of S100B Master Standard, Quality Controls and Wash Solution Concentrate.

Wash Solution

Dilute 100 mL of Wash Solution Concentrate with 900 mL of deionized (distilled) water.

Stability and storage: The diluted Wash Solution is stable for one month when stored at 2-8°C.

S100B Standards

Reconstitute S100B Master Standard with 100 µL of distilled water. **Add 1200 µL of Dilution Buffer**.
Shake gently for 25-30 minutes (not to foam).
 The concentration of the S100B in the stock solution is 4000 pg/mL. Prepare Standard Solutions using Dilution Buffer as follows:

Standard volume	Dilution Buffer	Concentration
300 µL of stock (4000 pg/mL)	300 µL	2000 pg/mL
300 µL of std. 2000 pg/mL	300 µL	1000 pg/mL
300 µL of std. 1000 pg/mL	300 µL	500 pg/mL
200 µL of std. 500 pg/mL	300 µL	200 pg/mL
300 µL of std. 200 pg/mL	300 µL	100 pg/mL
300 µL of std. 100 pg/mL	300 µL	50 pg/mL

Dilute prepared Standard Solutions **1:4** with Dilution Buffer prior to use in ELISA, e.g. 100 µL standard solution + 300 µL Dilution Buffer for duplicates.
Do not store the diluted standard solutions.

Stability and storage:

Reconstituted and undiluted Standard Solutions should be frozen at –20°C until next use.

Do not store the diluted (1:4) standard solutions.

Controls

Dissolve lyophilized Quality Controls with 250 µL of distilled water to the original volume and shake carefully (not to foam). Let stand for 25-30 minutes. These solutions are prepared for subsequent diluting.

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Dilute Quality Controls prior to use **1:4** with Dilution Buffer, e.g. 60 µL sample + 180 µL Dilution Buffer when assaying samples in singlets or preferably in duplicates.

Stability and storage:

Undiluted Quality Controls should be frozen at –20°C until next use.

Avoid repeated freezing of dissolved Controls. Do not store the diluted (4x) Controls.

9   PREPARATION OF SAMPLES

Dilute serum, heparin plasma or cerebrospinal fluid samples prior to use **1:4** with Dilution Buffer,
e.g. 60 µL sample + 180 µL Dilution Buffer when assaying samples in singlets or preferably in duplicates.

10 ASSAY PROCEDURE

1. Pipette 100 µL of diluted Standards, Controls and samples, preferably in duplicates, into the appropriate wells. Pipette 100 µL of Dilution Buffer as Blank in the wells. (See Figure 1 for example of work sheet.)

2. **Incubate** the plate **at room temperature (ca. 25°C) for 90 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

3. Wash the wells 3-times with Wash Solution (0.35 mL per well).

4. Add 100 µL of Conjugate Solution.

5. **Incubate** the plate **at room temperature (ca. 25°C) for 90 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

6. Wash the wells **5-times** with Wash Solution (0.35 mL per well).

7. Add 100 µL of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)

8. **Incubate** the plate for **10 minutes at room temperature**. (The incubation time may be extended [up to 30 minutes] if the reaction temperature is below than 20°C.) No shaking!

9. Stop the colour development by adding 100 µL of Stop Solution.

10. Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 5 minutes following step 9.)

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine S100B concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

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	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 2000	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 1000	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 500	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 200	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 100	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 50	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC High	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of work sheet.

11 CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of standards versus log of the known concentration (X) of standards, using the four-parameter function. Results are reported as concentration of S100B (pg/mL) in samples.

Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of standards).

Samples, Quality Controls and Standards are diluted 1:4 prior to analysis, so there is no need to account for this dilution.

12 LIMITS OF ASSAY

Results exceeding S100B levels of 2000 pg/mL should be repeated with more diluted samples. Dilution factors need to be taken into consideration in calculating the S100B concentration in this case.

13 PERFORMANCE CHARACTERISTICS

Typical analytical data obtained with the Human S100B ELISA are presented in this chapter.

For actual Standard Curve and Quality Controls values see the Certificate of Analysis.

13.1 Sensitivity

The limit of detection (defined as human S100B concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:

<u>Analytical Limit of Detection</u> is calculated from the real S100B values in wells and is 5 pg/mL.

<u>Assay Sensitivity</u> takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 5 pg/mL x 4 = 20 pg/mL

Dilution Buffer is pipetted into blank wells.

13.2   Specificity

The antibodies in Human S100B ELISA kit are highly specific for human S100B with no detectable crossreactivities to human S100P, S100Z, neuroglobin and GFAP.

13.3   Precision

Intra-assay (Within-Run) (n=7)

Sample	Mean (pg/ mL)	Standard Deviation (pg/ mL)	CV (%)
1	410.9	18.5	4.50
2	935.8	37.8	4.04

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (pg/ mL)	Standard Deviation (pg/ mL)	CV (%)
1	143.6	6.90	4.0
2	416.9	26.18	5.2
3	474.5	17.84	3.1

13.4   Spiking Recovery

Serum samples were spiked with different amounts of S100B antigen and assayed.

Sample	Observed (pg/ mL)	Expected (pg/ mL)	Recovery O/E (%)
1	150.7	-	-
	282.9	250.7	112.8
	358.1	350.7	102.1
	594.7	550.7	108.0
2	586.9	-	-
	715.3	686.9	104.1
	895.1	786.9	113.8
	1100.6	986.9	111.5
3	419.0	-	-
	557.8	519.0	107.5
	693.2	619.0	112.0
	928.8	819.0	113.4

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13.5 Linearity

Diluted serum samples (1:4) were subsequently diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ mL)	Expected (pg/ mL)	Recovery O/E (%)
1	-	618.8	-	-
	2x	284.5	309.4	92.0
	4x	158.4	154.7	102.4
	8x	74.1	77.4	95.8
2	-	1266.2	-	-
	2x	610.0	633.1	96.4
	4x	312.4	316.6	98.7
	8x	161.3	158.3	101.9
3	-	1530.8	-	-
	2x	862.8	765.4	112.7
	4x	418.2	382.7	109.3
	8x	210.9	191.4	110.2

14 DEFINITION OF S100B MASTER STANDARD

A S100B (ββ homodimer) protein purified from human brain tissue is used as the standard in this assay. S100B is a 21 kDa protein.

15 TROUBLESHOOTING AND FAQS

1.   Weak signal in all wells

Possible explanations:

− Omission of a reagent or a step

− Improper preparation or storage of a reagent

− Assay performed before reagents were allowed to come to room temperature

2.   High signal and background in all wells

Possible explanations:

− Improper or inadequate washing

− Overdeveloping; incubation time should be decreased before addition of Stop Solution

3.   High coefficient of variation (CV)

Possible explanation:

− Improper or inadequate washing

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4.   Clinical relevance

Serum samples and cerebrospinal fluids were taken from 8 patients with head injury or brain disorder and measured in the assay, results shown below:

Volunteer No.	Serum (pg/mL)	Cerebrospinal fluid (pg/mL)
1	95.7	394
2	52.3	2194
3	31.8	485
4	42.6	479
5	0	237
6	0	890
7	0	575
8	0	893

5.   Effect of freezing/thawing on the concentration of Human S100B in samples

The concentration of S100B in serum and cerebrospinal fluid samples is decreased after repeated (3x) freezing/thawing cycles.

Sample	Number of freezing/ thawing cycles	Concentration of S100B in cerebrospinal fluid (pg/mL)
1	1x	221.3
	3x	132.1
	5x	93.1
2	1x	218.8
	3x	191.5
	5x	174.3
3	1x	304.8
	3x	203.7
	5x	191.0

6.   Stability of samples at 4°C

Samples should be stored at –20°C. However, no decline was observed in concentration of S100B in serum and cerebrospinal fluid samples when stored at 4°C for 2 weeks. To avoid microbial contamination, add NaN_3 to a final concentration 0.1% to the samples.

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S amp le	Incubation: Temperature Period	Concentration of S100B in cerebrospinal fluid (pg/mL)
1	- 20 °C	148.3
	4 °C, 1 day	142.9
	4 °C, 7 day	141.7
	4 °C, 14 days	149.2
2	- 20 °C	190.3
	4 °C, 1 day	202.5
	4 °C, 7 day	196.4
	4 °C, 14 days	188.2
3	- 20 °C	183.1
	4 °C, 1 day	183.1
	4 °C, 7 day	168.3
	4 °C, 14 days	172.8

7.   Why are standard solutions diluted 1:4 prior to use in ELISA and what does it mean for calculation of results?

The dilution buffer suppresses the matrix effect of samples. It is a common practice to use the same dilution for samples and standards because of simple calculation of results: concentrations of samples can be read directly off the curve without considering a dilution factor. Thus the same dilution 1:4 is used for standards, controls and serum/plasma samples in Human S100B ELISA.

Samples exceeding human S100B level of 2000 pg/mL should be measured at higher degree of dilution (e.g. 1:16) and dilution factors need to be taken into consideration when calculating human S100B concentrations then (the dilution factor is 4 in this case).

16 REFERENCES

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SYMBOLS USED WITH DRG ASSAY´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..