Human/Mouse/Rat S100B Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00585

Size: 96T

Sensitivity: 17.3 pg/mL

Range: 125-8000 pg/mL

Usage: For the quantitative detection of human/mouse/rat S100B concentrations in tissue lysate and human cerebrospinal fluid (CSF).

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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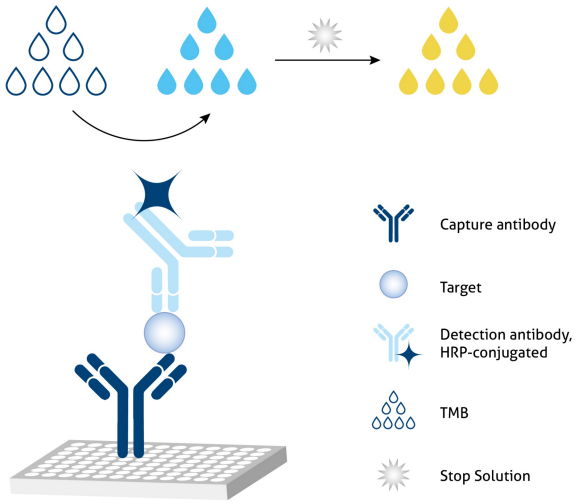
1. Background

S100B is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs and has been implicated in the regulation of cellular activities such as metabolism, motility and proliferation. S100B is highly expressed in astrocytes in the brain. It is also expressed in the Schwann cells of the peripheral nervous system, and outside the nervous system in melanocytes, adipocytes and chondrocyte. Elevated S100B levels in cerebrospinal fluid and serum reported after head trauma, subarachnoid hemorrhage, and stroke were correlated with the extent of brain damage. An association between elevated serum S100B and the presence of brain metastases has been reported. Increased S100B levels may also be found in patients with cerebral small vessel disease (SVD).

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 16000 pg/bottle; lyophilized	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1 - 30 mL/bottle	2 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

6.2 Human Cerebrospinal Fluid (CSF): Collect CSF samples in a tube and centrifuge for 15 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

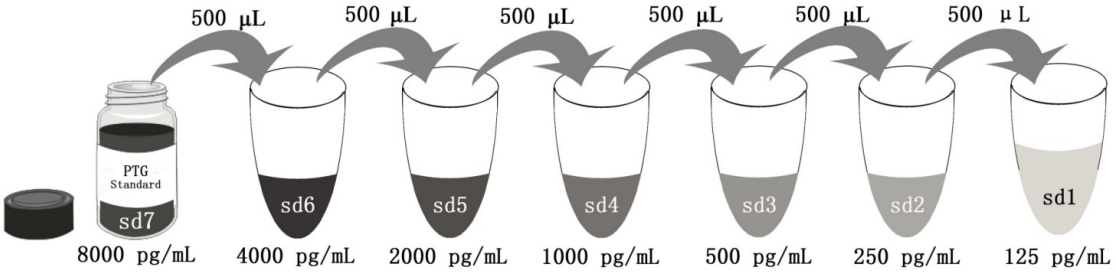
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated for a few seconds prior to use).

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:1,000 to 1:16,000 is recommended for tissue lysate; 1:4 or 1:8 is recommended for human cerebrospinal fluid (CSF).

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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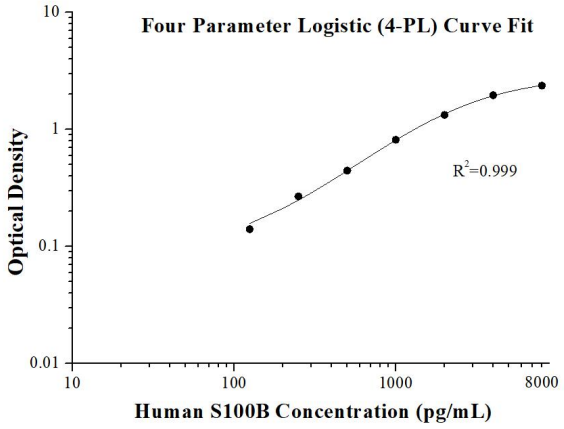
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0873 0.0683	0.0778	-
125	0.2317 0.2053	0.2185	0.1407
250	0.3495 0.3418	0.34565	0.26785
500	0.5288 0.5176	0.5232	0.4454
1000	0.899 0.8906	0.8948	0.817
2000	1.4215 1.4011	1.4113	1.3335
4000	2.0723 2.0117	2.042	1.9642
8000	2.4761 2.4223	2.4492	2.3714

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	2,066.1	160.4	7.8
2	8	979.2	52.8	5.4
3	8	129.6	12.2	9.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	2,209.1	208.6	9.4
2	16	923.8	79.9	8.7
3	16	137.7	14.7	10.6

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9.3 Recovery

The recovery of human/mouse/rat S100B spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Tissue lysate	1:4,000	89	85-94
Human cerebrospinal fluid (CSF)	1:16	109	91-127
	1:32	93	89-96

9.4 Sample values

Tissue lysate

	S100B (**ng/mL**)	Total protein (**mg/mL**)
Mouse brain tissue lysate	4,145.6	2.2
Rat brain tissue lysate	28,511.1	1.6

Human cerebrospinal fluid (CSF) - Human cerebrospinal fluid (CSF) samples were evaluated for the presence of S100B in this assay, and measured 5863.1 pg/mL.

9.5 Sensitivity

The minimum detectable dose of human/mouse/rat S100B is 3.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The tissue lysate was initially diluted 1:4,000. The human cerebrospinal fluid (CSF) was initially diluted 1:2.)

		Tissue lysate	Human cerebrospinal fluid (CSF)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	104	109
	Range (%)	102-106	108-110
1:8	Average% of Expected	103	119
	Range (%)	99-107	118-120
1:16	Average% of Expected	110	118
	Range (%)	109-111	106-129

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9.7 Specificity

This assay recognizes natural and recombinant human/mouse/rat S100B.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

APRIL

TNF-α

TNF-β

TRAIL

10. References

1. Donato, R. The international journal of biochemistry & cell biology vol. 33,7 (2001): 637-68.

2. Heizmann, Claus W et al. Frontiers in bioscience : a journal and virtual library vol. 7 (2002): d1356-68.

3. Kanner, Andrew A et al. Cancer vol. 97,11 (2003): 2806-13.

4. Vogelbaum, Michael A et al. Cancer vol. 104,4 (2005): 817-24.

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