User Guide

Human S100B ELISA Kit

96-Well Plate

EZHS100B-33K

Intended Use

S100B is a calcium-binding protein physiologically produced mainly by astrocytes in the central nervous system and has been implicated in the development and maintenance of the nervous system. Although the mechanism of S100B secretion is unknown, it appears to be affected by oxidative stress. At nanomolar concentrations (normal levels), S100B is able to protect neurons against glutamate toxicity. Glial damage or astrocytic reactions to neural injury (reactive astrogliosis) causes an increase in S100B to micromolar concentrations. High levels of S100B will further increase neuronal damage. High levels of S100B are considered a biomarker that could indicate damage or dysfunction of CNS.

For Research Use Only. Not for Use in Diagnostic Procedures.

This kit requires 50 µL sample volume.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of S100B molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-S100B monoclonal antibody
- Wash-away of unbound materials from samples
- Binding of a biotinylated anti-S100B polyclonal antibody to the captured molecules
- Wash-away of unbound materials from samples
- Conjugation of horseradish peroxidase to the immobilized biotinylated antibodies
- Wash-away of free enzyme conjugates
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3’,5,5’-tetramethylbenzidine

The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured S100B in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of S100B.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Volume	Quantity	Catalogue Number
S100B ELISA Plate with Adhesive Plate Sealer	-	1 Strip Plate 2 sheets	EP33
10X HRP Wash Buffer Concentrate	50 mL	2 bottles	EWB-HRP
S100B Standard	0.5 mL Lyophilized	1 vial	E8033-K
S100B Quality Controls 1 and 2	0.5 mL/vial Lyophilized	2 vials	E6033-K
Matrix Solution	6 mL Lyophilized	1 EA	EMTX-HS
Assay Buffer	30 mL	1 bottle	AB-33K
S100B Detection Antibody	12 mL	1 bottle	E1033
Enzyme Solution	12 mL	1 bottle	EHRP
Substrate	12 mL	1 bottle	ESS-TMB
Stop Solution	12 mL	1 bottle	ET-TMB

Storage and Stability

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze thaws should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

GHS Hazardous Labels

Ingredient	Cat. No.	Full Label	
Human S100B Quality Controls 1 and 2	E6033-K		**Warning:** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Human S100B Standard	E8033-K		**Warning:** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment
Stop Solution	ET-TMB		**Warning:** May be corrosive to metals.
Wash Buffer Concentrate	EWB-HRP		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Matrix Solution	EMTX-HS	No symbol required	**Warning:** Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Reagent Precautions

Sodium Azide

Sodium azide has been added to certain reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium azide may react with lead and copper plumbing to form explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Hydrochloric Acid

Hydrochloric acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

Materials Required

(Not Provided)

- Multi-channel pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- Distilled, De-ionized Water
- Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

Sample Collection and Storage

This kit requires 50 µL sample volume. For serum samples, 50 µL of neat sample volume may be used. For cerebrospinal fluid (CSF), sample will need to be diluted 1:10 in Assay Buffer.

1. To prepare serum, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ± 2 °C.
3. Transfer and aliquot serum samples in separate tubes of small quantity. Date and identify each sample.
4. Use freshly prepared serum or store samples at −20 ± 5 °C for later use. Avoid multiple freeze/thaw cycles. Repeated freeze/thaw cycles will result in inaccurate measurement of sample concentration.
5. Avoid using samples with gross hemolysis or lipemia.
6. CSF samples should be assayed immediately after collection or should be stored in small aliquots at −20 °C to avoid repeated freeze/thaw cycles.

7. Insoluble material should be removed from all samples by centrifugation (4000 x *g* for 10 minutes at 4 °C) before testing.

8. Samples should be kept on ice for the duration of the assay set up procedure. For better result the samples should be loaded in the plate within 10-15 minutes.

9. Customers need to determine the optimal dilution factor for their samples. Generally, serum samples should be used as neat and CSF samples should be diluted 1:10 using the Assay Buffer provided in the kit as the sample diluent (For example, add 12 µL sample to 108 µL Assay Buffer). This dilution should occur immediately before loading the sample on the plate.

10. This assay can also be used on plasma samples, but it is recommended to use heparin as the anti-coagulant. If using EDTA as the anti-coagulant the effect on the assay outcome at the dose of K2EDTA greater than 1.8 mg/mL, or K3EDTA greater than 1.5 mg/mL should be pre-determined.

Reagent Preparation

S100B Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the S100B Standard with 0.5 mL distilled or de-ionized water to give a concentration described on the analysis sheet included in the kit. Invert and vortex until completely in solution.

2. Label six tubes as 1, 2, 3, 4, 5 and 6. Add 200 µL Assay Buffer to each of the six tubes. Perform 3 times serial dilutions by adding 100 µL of the reconstituted standard to Tube 6, mix well and transfer 100 µL from Tube 6 to Tube 5, mix well and transfer 100 µL from Tube 5 to Tube 4, mix well and transfer 100 µL from Tube 4 to Tube 3, mix well and transfer 100 µL from Tube 3 to Tube 2, mix well and transfer 100 µL from Tube 2 to Tube 1. Mix well.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration (pg/mL)
0.5 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration or dilution factor(pg/mL)
Tube 6	0.2 mL	0.1 mL of reconstituted standard	X/3
Tube 5	0.2 mL	0.1 mL of Tube 6	X/9
Tube 4	0.2 mL	0.1 mL of Tube 5	X/27
Tube 3	0.2 mL	0.1 mL of Tube 4	X/81
Tube 2	0.2 mL	0.1 mL of Tube 3	X/243
Tube 1	0.2 mL	0.1 mL of Tube 2	X/729

S100B Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each S100B Quality Control 1 and Quality Control 2 with 0.50 mL distilled or de-ionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ −20 °C.
Avoid further freeze/thaw cycles.

Preparation of Matrix Solution

Add 6 mL Assay Buffer to the bottle containing lyophilized Matrix Solution. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ −20 °C for up to one month.

S100B ELISA Assay Procedure

Pre-warm all reagents to room temperature before setting up the assay.

1. Dilute the 10X concentrated HRP Wash Buffer 10-fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder and fill each well with 300 µL diluted Wash Buffer. Decant Wash Buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add in 50 µL Matrix Solution to Blank, Standards, Quality Control wells and CSF sample wells. (Note: please vortex well all the reagents before adding into the wells including assay buffer and matrix solution. Refer to Microtiter Plate Arrangement for suggested sample order placement.)

4. Add in 50 µL Assay Buffer to Serum and/or Plasma sample wells.

5. Add in duplicate 50 µL Assay Buffer to blank wells.

6. Add in duplicate 50 µL human S100B standards in the order of ascending concentration to the appropriate wells.

7. Add in duplicate 50 µL QC1 and 50 µL QC2 to the appropriate wells.

8. Add in duplicate 50 µL CSF, serum or plasma samples to the appropriate wells.

9. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 500 to 600 rpm.

10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well. Wash wells 5 times with diluted Wash Buffer, 300 µL per well, per wash. Decant and tap after each wash to remove residual buffer.

11. Add 100 µL Detection Antibody solution to each well. Re-cover plate with sealer and incubate at room temperature for 1.5 hours on an orbital micro-titer plate shaker set to rotate at moderate speed, approximately 500 to 600 rpm.

12. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well. Wash wells 5 times with diluted Wash Buffer, 300 µL per well, per wash. Decant and tap after each wash to remove residual buffer.

13. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking (500 to 600 rpm) at room temperature for 30 minutes on the micro-titer plate shaker.

14. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid. Wash wells 5 times with diluted Wash Buffer, 300 µL per well, per wash. Decant and tap after each wash to remove residual buffer.

15. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5 to 25 minutes. Blue color should be formed in wells of the S100B standards with intensity proportional to increasing concentrations of S100B.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

16. Remove sealer and add 100 µL Stop Solution (**Caution:** Corrosive Solution) and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units.

Assay Procedure for Human S100B ELISA Kit

Well #	Step 1	Step 2	Step 3	Step 4-5	Step 6-8	Step 9-10	Step 11	Step 12	Step 13	Step 14	Step 15-16		
			Matrix Solution	Assay Buffer	Standards/ QCs/ Samples		Detection Antibody		Enzyme Solution		Substrate		Stop solution
A1, B1	Dilute both bottles of 10X HRP Wash Buffer with 900 mL deionized water	Wash plate 3X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	50 µL	50 µL	-	Seal, Agitate, Incubate 2 hours at Room Temperature Wash 5X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 1.5 hours at Room Temperature. Wash 5X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, incubate 30minutes at Room Temperature. Wash 5 X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate for 5-25 minutes at Room Temperature.	100 µL
C1, D1			50 µL	-	50 µL of Tube 1								
E1, F1			50 µL	-	50 µL of Tube 2								
G1, H1			50 µL	-	50 µL of Tube 3								
A2, B2			50 µL	-	50 µL of Tube 4								
C2, D2			50 µL	-	50 µL of Tube 5								
E2, F2			50 µL	-	50 µL of Tube 6								
G2, H2			50 µL	-	50 µL of Reconstituted Standard								
A3, B3			50 µL	-	50 µL of QC 1								
C3, D3			50 µL	-	50 µL of QC 2								
E3, F3 etc.			50 µL	No buffer	50 µL of CSF Sample								Read Absorbance at 450 nm and 590 nm.
			OR										
			No matrix	50 µL	50 µL of Serum/Plasma Sample								

Microtiter Plate Arrangement

S100B ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 4 Std	QC 1									
B	Blank	Tube 4 Std	QC 1									
C	Tube 1 Std	Tube 5 Std	QC 2									
D	Tube 1 Std	Tube 5 Std	QC 2									
E	Tube 2 Std	Tube 6 Std	Sample 1									
F	Tube 2 Std	Tube 6 Std	Sample 1									
G	Tube 3 Std	Reconstituted Standard	Sample 2									
H	Tube 3 Std	Reconstituted Standard	Sample 2									

Calculations

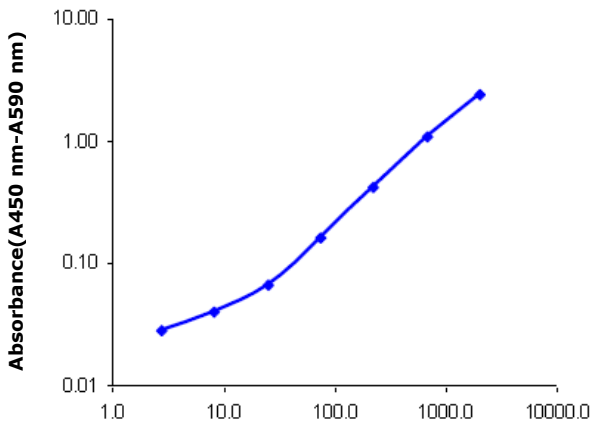
The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function.

Interpretation

- The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
- If the difference between duplicate results of a sample is > 15% CV, repeat the sample.
- The limit of sensitivity of this assay is 2.7 pg/mL S100B (50 µL sample size).
- The approximate range of this assay is 2.7 pg/mL to 2000 pg/mL S100B 50 µL sample size). Any result greater than 2000 pg/mL in a 50 µL sample should be diluted using Assay Buffer and repeated until the results fall within range.

Graph of Typical Reference Curve

Human S100B ELISA Standard Curve



Typical Standard Curve, not to be used to calculate data.

Assay Characteristics

Sensitivity

The lowest level of S100B that can be detected by this assay is 2.7 pg/mL using a 50 µL sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 12) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

Specificity

The antibody pair used in this assay measures Human S100B and has no cross-reactivity with S100A1, S100A6 and S100A13.

Precision

The assay variations of our S100B ELISA kits were studied on two samples at two levels on the S100B standard curve. The mean intra-assay variation was calculated from results of twenty-four determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of six separate assays with duplicate samples in each assay.

Intra-Assay Variation

	Mean S100B Levels (pg/mL)	Intra-Assay %CV
1	26.9	4.8
2	278.5	2.9

Inter-Assay Variation

	Mean S100B Levels (pg/mL)	Inter-Assay %CV
1	28.5	4.4
2	280	1.9

The assay variations of S100B ELISA kits were studied on two samples at two levels on the S100B standard curve. The mean intra-assay variation was calculated from results of twenty-four determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of six separate assays with duplicate samples in each assay.

Spike Recovery of Human S100B in Assay Samples

Spike recovery of human S100B in CSF

Sample	S100B Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% of Recovery
1	0	36.1	-	-
	25	61	58.1	95
	74	110	104.3	95
	222	258	239.4	93
2	0	96.8	-	-
	25	122	115.8	95
	74	171	164.4	96
	222	319	307.5	96
3	0	31.1	-	-
	25	56	51.6	92
	74	105	95.4	91
	222	253	227	90
4	0	39.4	39.4	-
	25	64	61.2	95
	74	113	106.8	94
	222	261	251.1	96
5	0	31.7	31.7	-
	25	57	52.6	93
	74	106	97.9	93
	222	254	239.8	95
Average	-	-	-	94

Spike recovery of human S100B in serum

Sample	S100B Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% of Recovery
1	0	29.4	-	-
	25	54	50.3	92
	74	103	94.8	92
	222	251	239.7	95
2	0	13.8	-	-
	25	39	35.5	91
	74	88	84	96
	222	236	231.6	98
3	0	18.1	-	-
	25	43	38.5	89
	74	92	77.1	84
	222	240	204.5	85
4	0	14.8	-	-
	25	40	36.5	92
	74	89	86.2	97
	222	237	236.8	100
5	0	11	-	-
	25	36	34	94
	74	85	78.7	93
	222	233	222.3	95
Average	-	-	-	93

Linearity of Sample Dilution

Effect of CSF Dilution

Sample	Volume (µL)	Expected (pg/mL)	Observed (pg/mL)	% of Expected
1	5	35.5	35.1	100
	2.5	-	18.2	104
	1.25	-	9.4	107
	0.625	-	5.9	134
2	5	90.1	90.1	100
	2.5	-	46.9	104
	1.25	-	24.7	110
	0.625	-	13.9	123
3	5	28.7	28.7	100
	2.5	-	15.2	106
	1.25	-	8.7	121
	0.625	-	4.9	137
4	5	36.7	36.7	100
	2.5	-	19.1	104
	1.25	-	10.7	117
	0.625	-	6.2	135
5	5	32.4	32.4	100
	2.5	-	15.6	96
	1.25	-	8.1	100
	0.625	-	5.2	128
Average	-	-	-	115

Effect of Serum Dilution

Sample	Volume (µL)	Expected (pg/mL)	Observed (pg/mL)	% of Expected
1	50	29.9	29.9	100
	25	-	15.2	102
	12.5	-	8.2	110
	6.25	-	4.1	110
2	50	15.2	15.2	100
	25	-	6.8	89
	12.5	-	3.7	97
	6.25	-	2	105
3	50	16.7	16.7	100
	25	-	8.6	103
	12.5	-	4.6	110
	6.25	-	2.4	115
4	50	12.9	12.9	100
	25	-	5.9	91
	12.5	-	3.2	99
	6.25	-	1.5	93
5	50	10.5	10.5	100
	25	-	4.1	78
	12.5	-	2	76
	6.25	-	1.1	84
Average	-	-	-	98

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results, the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started, all steps should be completed with precise timing and without interruption.
- Avoid cross-contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the wells is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High absorbance in background or blank wells could be due to:
    - cross well contamination by standard solution or sample or
    - washing of wells with HRP Wash Buffer or
    - overexposure to light after substrate has been added.

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
Human S100B ELISA Plate	EP33
10X HRP Wash Buffer Concentrate	EWB-HRP
S100B ELISA Standard	E8033-K
S100B Quality Controls 1 and 2	E6033-K
Matrix Solution	EMTX-HS
Assay Buffer	AB-33K
S100B ELISA Detection Antibody	E1033
Enzyme Solution	EHRP
Substrate Solution	ESS-TMB
Stop Solution	ET-TMB

Notice

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