KAMIYA BIOMEDICAL COMPANY

S-100β ELISA

For the quantitative determination of S-100β in human, mouse and rat plasma.

Cat. No. KT-435

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

S-100β ELISA
Cat. No. KT-435

INTENDED USE
The S-100β ELISA is for the quantitative determination of S-100β in human, mouse and rat plasma. For research use only.

INTRODUCTION
S-100 protein has a molecular weight of 21K Dalton and consists of two subunits, α chain and β chain. It is known that combination of these subunits is different from the location in human body. S-100ββ is localized in glial cell and schwann cell, S-100αβ in glial cell and S-100αα in striated muscle, heart and kidney.
It was reported that the concentration of S-100β in cerebrospinal fluid was a useful marker for studying the degree of brain damage after head injury, cerebral hemorrhage and ischemic stroke. Recently another report described that the increasing of S-100β in blood correlated to the degree of brain damage after cerebral ischemia, infarction, hemorrhage and severe head injury.

PRINCIPLE
This ELISA kit for determination of S-100β in plasma samples is based on a sandwich enzyme immunoassay. To the wells of plate coated with highly purified antibody against S-100β, calibrators or samples are added for the 1st step immunoreaction. After the 1st step incubation and plate washing, labeled antibody solution (biotinylated rabbit anti-bovine S-100β polyclonal antibody) is added as the 2nd step to form antibody-antigen-labeled antibody complex on the surface of the wells. After the 2nd step incubation and rinsing out excess labeled antibody, horseradish peroxidase (HRP) labeled streptoavidin (SA) is added for binding to labeled antibody. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of S-100β is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	Microtiter plate	1 plate (96 wells)	Rabbit anti-bovine S-100ß
2. S-100β Calibrator	Lyophilized	1 vial (5 ng)	Bovine S-100β
3. Labeled antibody	Liquid	1 bottle (11 mL)	Biotinylated rabbit anti-bovine S-100β antibody
4. SA-HRP solution	Liquid	1 bottle (11 mL)	HRP labeled streptoavidin
5. Substrate buffer	Liquid	1 bottle (26 mL)	Citrate buffer containing 0.015% hydrogen peroxide
6. OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7. Stop solution	Liquid	1 bottle (11 mL)	1M H_2SO_4
8. Buffer solution	Liquid	1 bottle (25 mL)	Phosphate buffer
9. Wash solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		4 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED
- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Microtiter plate shaker
- Washing device for microtiter plate, dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Glass test tubes for preparation of calibrator solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

NOTES

1. EDTA-2Na (1 mg/mL) additive blood collection tubes are recommended for the plasma sample collection. It is strongly recommended that plasma samples should be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amounts and frozen at or below -30℃ and thawed before assay. Avoid repeated freezing and thawing of samples.
2. S-100ß calibrator and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted calibrator in glass vials or tubes should be stored at 4℃ (stable for 2 weeks). It is also possible to keep calibrator stable for 4 weeks if calibrator is stored at or below –30℃.
3. During storage of wash solution (concentrated) at 4℃, precipitates may be observed, however they will dissolve when diluted.
4. When concentration of S-100ß in samples is expected to exceed 5 ng/mL, the sample needs to be diluted with buffer solution to a proper concentration.
5. Read optical absorbance of solution in the wells immediately after stopping color reaction.
6. Perform all the determinations in duplicate.
7. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
8. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.
9. Pipetting operations may affect the precision of the assay. Pipette calibrator solutions or samples into each well of plate precisely. In addition, use clean test tubes or vessels in assay and use a new tip for each calibrator or sample to avoid cross contamination.
10. To quantitate accurately, always run a calibration curve when testing samples.
11. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 minutes.
12. During incubation except the color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction (approximately 100 rpm).

REAGENT PREPARATION

1. Preparation of the calibrators:
   Reconstitute the S-100β calibrator (lyophilized 5 ng/vial) with 1 mL of buffer solution, which makes a 5 ng/mL calibrator solution. The reconstituted calibrator solution (0.2 mL) is diluted with 0.2 mL of buffer solution which yields a 2.5 ng/mL calibrator solution. Repeat the dilution procedure to make each of 1.25, 0.625, 0.313, 0.156 and 0.078 ng/mL calibrator solutions. Buffer solution itself is used as 0 ng/mL.

2. Preparation of the substrate solution:
   Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

3. Preparation of the wash solution:
   Dilute 50 mL of the wash solution (concentrated) to 1,000 mL with distilled or de-ionized water.

4. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

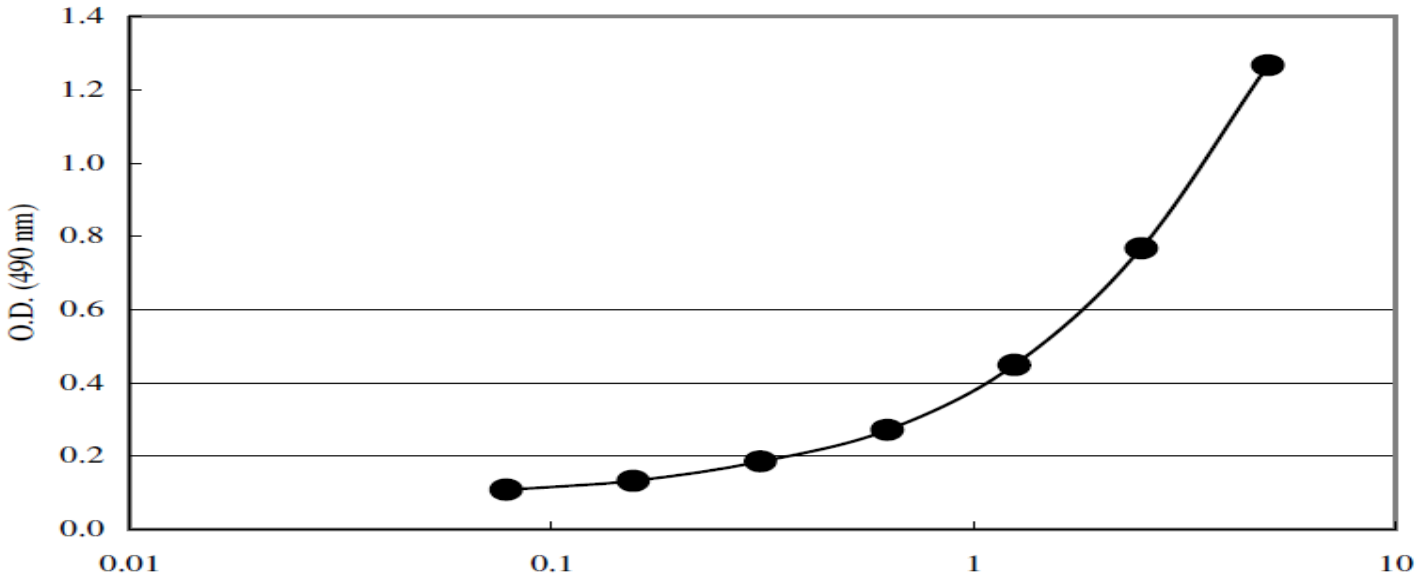
PROCEDURE

1. Bring all the reagents to room temperature (20-30°C) before starting assay.
2. Fill 0.30 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 100 µL of buffer solution into all of the wells first, then introduce 20 µL each of calibrator solution (0, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5 ng/mL) or samples into the wells. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 minutes.
4. Cover the plate with a Plate Seal and incubate it at room temperature (20-30°C) for 3 hours. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)

5. Take off the Plate Seal, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
6. Add 100 µL of labeled antibody solution into the wells.
7. Cover the plate with Plate Seal and incubate it at room temperature (20-30℃) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)
8. Take off the Plate Seal, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
9. Pipette 100 µL of SA-HRP solution into the wells.
10. Cover with the Plate Seal and incubate the plate at room temperature (20-30℃) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)
11. Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
12. Take off the Plate Seal, aspirate and wash the wells 5 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
13. Add 100 µL of the substrate solution containing OPD into the wells, cover the plate with Plate Seal and keep it still for 30 minutes at room temperature (20-30℃) for color reaction.
14. Add 100 µL of the stop solution into the wells to stop color reaction.
15. Read the optical absorbance of the wells at 490 nm. The dose-response curve of this assay fits best to a 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. Otherwise calculate mean absorbance values of wells containing calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of calibrator; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this calibration curve.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

<Human plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.21		
0.10	0.30	0.31	96.77
0.50	0.67	0.71	94.37
2.00	1.91	2.21	86.43

<Human plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.18		
0.10	0.27	0.28	96.43
0.50	0.61	0.68	89.71
2.00	1.65	2.18	75.69

<Rat plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.55		
0.10	0.62	0.65	95.39
0.50	0.89	1.05	84.76
2.00	1.99	2.55	78.04

<Rat plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	1.03		
0.10	1.11	1.13	98.23
0.50	1.38	1.53	90.20
2.00	2.27	3.03	74.92

<Rat plasma C>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.45		
0.10	0.53	0.55	96.36
0.50	0.79	0.95	83.16
2.00	1.78	2.45	72.65

<Mouse plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.08		
0.10	0.16	0.18	88.89
0.50	0.53	0.58	91.38
2.00	1.81	2.08	87.02

<Mouse plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.11		
0.10	0.21	0.21	100.00
0.50	0.54	0.61	88.53
2.00	1.72	2.11	81.52

Precision and reproducibility

- Intra-assay CV (%): 2.99 – 4.82
- Inter-assay CV (%): 4.82 – 9.20

Assay Range

0.078 – 5 ng/mL

Cross-Reactivity

The ELISA kit shows 0.2% cross-reactivity to bovine S-100αα.

FOR RESEARCH USE ONLY

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4. SA-HRP solution	Liquid	1 bottle (11 mL)	HRP labeled streptoavidin
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9. Wash solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		4 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED
- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Microtiter plate shaker
- Washing device for microtiter plate, dispenser with aspiration system
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- Graduated cylinder (1,000 mL)
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NOTES

1. EDTA-2Na (1 mg/mL) additive blood collection tubes are recommended for the plasma sample collection. It is strongly recommended that plasma samples should be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amounts and frozen at or below -30℃ and thawed before assay. Avoid repeated freezing and thawing of samples.
2. S-100ß calibrator and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted calibrator in glass vials or tubes should be stored at 4℃ (stable for 2 weeks). It is also possible to keep calibrator stable for 4 weeks if calibrator is stored at or below –30℃.
3. During storage of wash solution (concentrated) at 4℃, precipitates may be observed, however they will dissolve when diluted.
4. When concentration of S-100ß in samples is expected to exceed 5 ng/mL, the sample needs to be diluted with buffer solution to a proper concentration.
5. Read optical absorbance of solution in the wells immediately after stopping color reaction.
6. Perform all the determinations in duplicate.
7. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
8. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.
9. Pipetting operations may affect the precision of the assay. Pipette calibrator solutions or samples into each well of plate precisely. In addition, use clean test tubes or vessels in assay and use a new tip for each calibrator or sample to avoid cross contamination.
10. To quantitate accurately, always run a calibration curve when testing samples.
11. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 minutes.
12. During incubation except the color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction (approximately 100 rpm).

REAGENT PREPARATION

1. Preparation of the calibrators:
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2. Preparation of the substrate solution:
   Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

3. Preparation of the wash solution:
   Dilute 50 mL of the wash solution (concentrated) to 1,000 mL with distilled or de-ionized water.

4. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

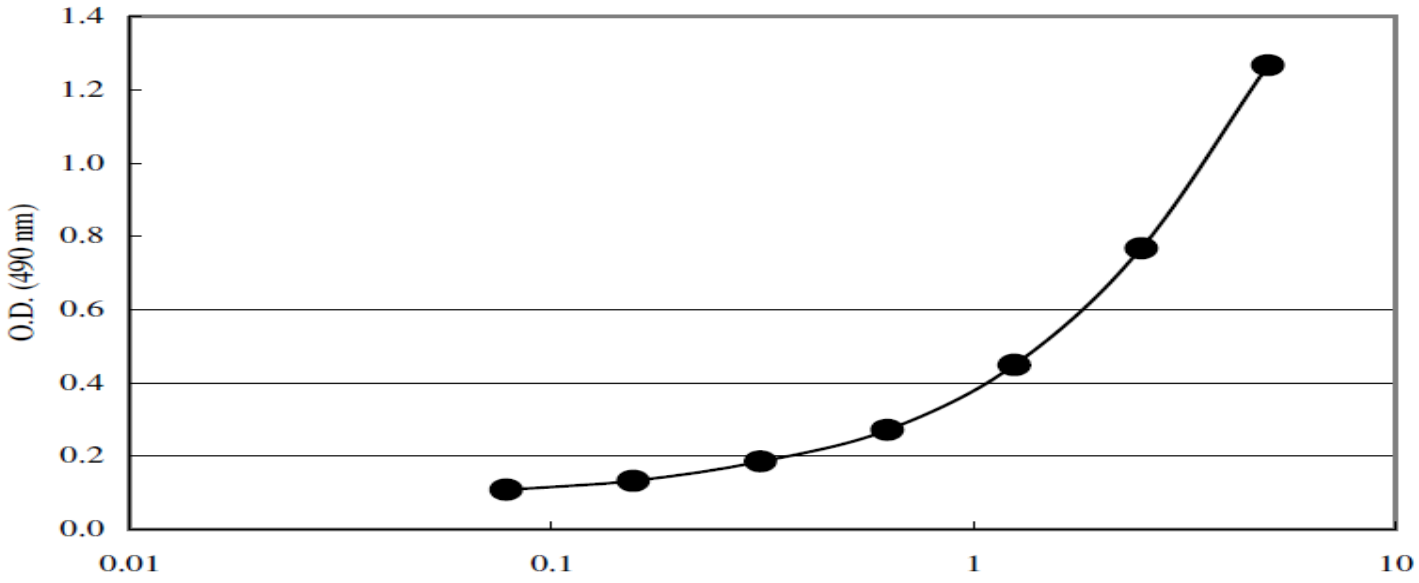
PROCEDURE

1. Bring all the reagents to room temperature (20-30°C) before starting assay.
2. Fill 0.30 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 100 µL of buffer solution into all of the wells first, then introduce 20 µL each of calibrator solution (0, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5 ng/mL) or samples into the wells. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 minutes.
4. Cover the plate with a Plate Seal and incubate it at room temperature (20-30°C) for 3 hours. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)

5. Take off the Plate Seal, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
6. Add 100 µL of labeled antibody solution into the wells.
7. Cover the plate with Plate Seal and incubate it at room temperature (20-30℃) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)
8. Take off the Plate Seal, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
9. Pipette 100 µL of SA-HRP solution into the wells.
10. Cover with the Plate Seal and incubate the plate at room temperature (20-30℃) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm)
11. Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
12. Take off the Plate Seal, aspirate and wash the wells 5 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
13. Add 100 µL of the substrate solution containing OPD into the wells, cover the plate with Plate Seal and keep it still for 30 minutes at room temperature (20-30℃) for color reaction.
14. Add 100 µL of the stop solution into the wells to stop color reaction.
15. Read the optical absorbance of the wells at 490 nm. The dose-response curve of this assay fits best to a 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. Otherwise calculate mean absorbance values of wells containing calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of calibrator; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this calibration curve.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

<Human plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.21		
0.10	0.30	0.31	96.77
0.50	0.67	0.71	94.37
2.00	1.91	2.21	86.43

<Human plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.18		
0.10	0.27	0.28	96.43
0.50	0.61	0.68	89.71
2.00	1.65	2.18	75.69

<Rat plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.55		
0.10	0.62	0.65	95.39
0.50	0.89	1.05	84.76
2.00	1.99	2.55	78.04

<Rat plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	1.03		
0.10	1.11	1.13	98.23
0.50	1.38	1.53	90.20
2.00	2.27	3.03	74.92

<Rat plasma C>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.45		
0.10	0.53	0.55	96.36
0.50	0.79	0.95	83.16
2.00	1.78	2.45	72.65

<Mouse plasma A>

Added Bovine S-100□□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.08		
0.10	0.16	0.18	88.89
0.50	0.53	0.58	91.38
2.00	1.81	2.08	87.02

<Mouse plasma B>

Added Bovine S-100□ (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.00	0.11		
0.10	0.21	0.21	100.00
0.50	0.54	0.61	88.53
2.00	1.72	2.11	81.52

Precision and reproducibility

- Intra-assay CV (%): 2.99 – 4.82
- Inter-assay CV (%): 4.82 – 9.20

Assay Range

0.078 – 5 ng/mL

Cross-Reactivity

The ELISA kit shows 0.2% cross-reactivity to bovine S-100αα.

FOR RESEARCH USE ONLY

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