YK151 S - 100β ELISA

FOR LABORATORY USE ONLY

＜Distributed by＞

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Contents

– Please read all the package insert carefully before beginning the assay –

YK151 S-100β ELISA Kit

Ⅰ. Introduction

S-100 protein has a molecular weight of 21K Dalton and is consist 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 an useful marker for diagnosis of 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.

YK151 S-100β ELISA Kit

- ▼ The assay kit can measure rat, mouse and human S-100β within the range of 0.078 - 5 ng/mL
- ▼ The assay is completed within 3+1+1h. + 0.5h.
- ▼ With one assay kit, 40 samples can be measured in duplicate
- ▼ Test sample: rat, mouse and human plasma
  Sample volume: 20 μL
- ▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) plasma 2.99 – 4.82
  Inter-assay CV (%) plasma 4.82 – 9.20
- ▼ Stability and storage
  Store all of the components at 2-8°C.
  This kit is stable under the condition for 9 months from the date of manufacturing.
  The expiry date is stated on the package

Contents

1) Antibody coated plate
2) S-100β standard
3) Labeled antibody solution
4) SA-HRP solution
5) Substrate buffer
6) OPD tablet
7) Stopping solution
8) Buffer solution
9) Washing solution (Concentrated)
10) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of S-100β in plasma sample. The kit is characterized by sensitive quantification and high specificity. In addition, it is not influenced by other components in plasma sample and needlessness of sample pre-treatment.

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

<Assay principle>

This kit for determination of S-100β in plasma sample is based on the sandwich enzyme immunoassay. During first immune incubation, S-100β in standards or in samples bind to the rabbit anti bovine S-100β antibody, which is coated on the surface of the microtiter plate. After incubation and plate washing, labeled antibody solution (biotinylated rabbit anti bovine S-100β polyclonal antibody) is added to bind to the antibody-antigen complex. Then, HRP labeled streptoavidin (SA-HRP) is added to form antibody-antigen-biotinylated antibody complex. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of S-100β is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiterplate	1 plate (96 wells)	Rabbit anti bovine S-100β
2.	S-100β Standard	Lyophilized	1 vial (5 ng)	Bovine S-100β
3.	Labeled antibody solution	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.	Stopping solution	Liquid	1 bottle (11 mL)	1M H_2SO_4
8.	Buffer solution	Liquid	1 bottle (20 mL)	Phosphate buffer
9.	Washing solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
10.	Adhesive foil		4 pieces	

Ⅳ. Method

<Equipment required>

1. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
2. Photometer for microtiter plate（plate reader）, which can read the extinction 2.5 at 490 nm
3. Microtiter plate shaker
4. Glass test tubes for preparation of standard solution
5. Washing device for microtiter plate, dispenser with aspiration system
6. Graduated cylinder（1,000 mL）
7. Distilled water or deionized water

<Preparatory work>

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

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

3. Preparation of the washing solution：
   Dilute 50 mL of the washing solution (concentrated) to 1,000 mL with distilled or deionized water.

4. Other reagents are ready for use.

<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 standard 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 standard solutions and samples for a whole plate should not exceed 30 minutes.

4. Cover the plate with adhesive foil 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 adhesive foil, aspirate and wash the wells 4 times with approximate 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 adhesive foil and incubate it at room temperature (20-30°C) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker. (approximately 100 rpm).

8. Take off the adhesive foil, aspirate and wash the wells 4 times with approximate 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. Add 100 μL of SA-HRP solution into the wells.

10. Cover with the adhesive foil and incubate the plate at room temperature (20-30°C) for 1 hour. During the incubation, the plate should be shaken with microtiter plate shaker. (approximately 100 rpm).

11. Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepare immediately before use.

12. Take off the adhesive foil, aspirate and wash the wells 5 times with approximate 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 adhesive foil and keep it still for 30 minutes at room temperature (20-30°C) for color reaction.

14. Add 100 μL of the stopping 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 standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Ⅴ. Notes

1. EDTA-2Na (1 mg/mL) additive blood collection tube is 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 amount and frozen at or below -30°C and thawing before assay. Avoid repeated freezing and thawing of samples.

2. S-100β standard**,** 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 standard in glass vials or tubes should be stored at 4°C (stable for 2 weeks). It is also possible to keep standard stable for 4 weeks if standard be stored at or below –30°C.

3. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 minutes.

4. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will dissolve when diluted.

5. Pipetting operations may affect the precision of the assay. Pipette standard solutions or samples into each well of plate precisely. In addition, use clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.

6. When concentration of S-100β in samples is expected to exceed 5 ng/mL, the sample needs to be diluted with buffer solution to a proper concentration.

7. During incubation except the color reaction, the plate should be shaken gently with a microtiter

plate shaker to promote immunoreaction. (approximately 100 rpm).

8. Perform all the determination in duplicate.

9. Read optical absorbance of solution in the wells immediately after stopping color reaction.

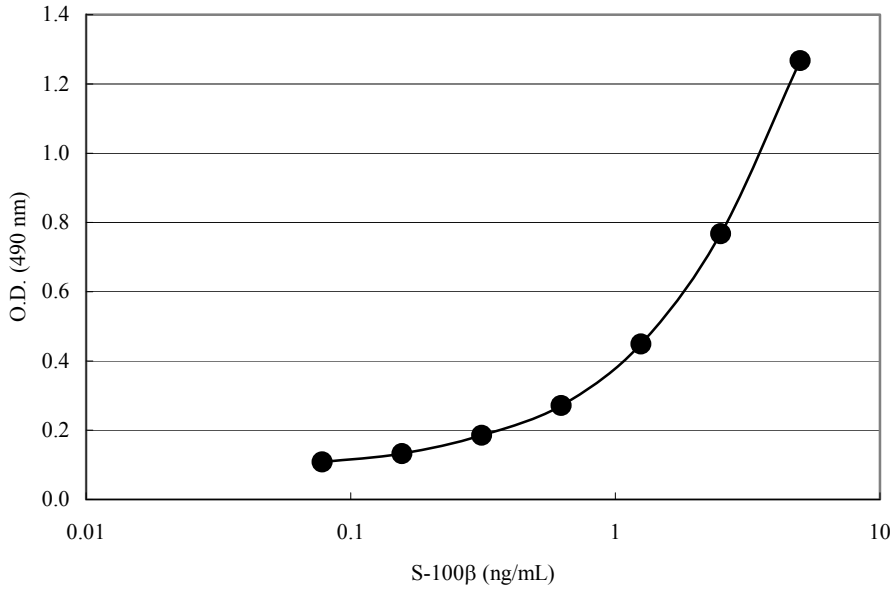
10. To quantitate accurately, always run a standard curve when testing samples.

11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

12. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.

Ⅵ. Performance Characteristics

Typical standard curve



<Analytical recovery>

<Human plasma>

<Human plasma A>

Added Bovine S-100β (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-100β (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>

<Rat plasma A>

Added Bovine S-100β (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-100β (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-100β (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>

<Mouse plasma A>

Added Bovine S-100β (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-100β (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

<Dilution test>

＜Human plasma＞

Human plasma	Dilution ratio	Observed (ng/mL)	Estimated (ng/mL)	Recovery (%)
Human plasma A	x 1.0	0.171		
	x 1.5	0.122	0.114	107.02
	x 2.0	0.088	0.086	102.33
Human plasma B	x 1.0	0.362		
	x 1.5	0.260	0.241	107.88
	x 2.0	0.187	0.181	103.31

＜Rat plasma＞

Rat plasma	Dilution ratio	Observed (ng/mL)	Estimated (ng/mL)	Recovery (%)
Rat plasma A	x 1.0	0.642		
	x 2.0	0.271	0.321	84.42
	x 4.0	0.110	0.161	68.32
Rat plasma B	x 1.0	1.211		
	x 2.0	0.524	0.606	86.47
	x 4.0	0.220	0.303	72.61
Rat plasma C	x 1.0	0.529		
	x 2.0	0.236	0.265	89.06
	x 4.0	0.094	0.132	71.21

＜Mouse plasma＞

Mouse plasma	Dilution ratio	Observed (ng/mL)	Estimated (ng/mL)	Recovery (%)
Mouse plsama A	x 1.0	0.104		
	x 1.5	0.062	0.069	89.86
	x 2.0	0.042	0.054	77.78
Mouse plasma B	x 1.0	0.154		
	x 1.5	0.098	0.102	96.08
	x 2.0	0.068	0.077	88.31

Precision and reproducibility

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

Ⅶ. Stability and Storage

<Storage> Store all of the components at 2-8°C.

<Shelf life> This kit is stable under the condition for 9 months from the date of manufacturing.
The expiry date is stated on the package.

<Package> For 96 tests per one kit.

Ⅷ. References

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