YK150　Human S-100β ELISA

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Contents

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

YK150 Human S-100β ELISA Kit

Ⅰ. Introduction

Human 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. And 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.

YK150 Human S-100β ELISA Kit

- ▼ The assay kit can measure S-100 β within the range of 98-6300 pg/mL
- ▼ The assay is completed within 15-24 hr.+4.5 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate
- ▼ Test sample: human plasma
  Sample volume: 30 μL
- ▼ The 96-well plate of this kit consists of 12 8-wells strips. So that divided use by the strips is possible at user's option.
- ▼ Precision and reproducibility
  Intra-assay CV (%) plasma 2.33 - 11.54
  Inter-assay CV (%) plasma 2.91 - 7.77
- ▼ Stability and storage
  Store all of the components at 2-8℃.
  This kit is stable under the condition for 5 months from the date of manufacturing.
  The expiry date is stated on the package.

Contents

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

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of human 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 1% cross reactivity to Human S-100αα and 74% to Human S-100αβ.

<Assay principle>
This ELISA kit for determination of human S-100β in plasma sample is based on the sandwich enzyme immunoassay. During first immune reaction, 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 (biotinylated rabbit anti bovine S-100β antibody) is added to bind to the antigen-antibody complex. Then, HRP labeled streptoavidin (SA-HRP) is added to form biotinylated rabbit anti bovine S-100-antigen-antibody complex. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of human S-100β is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiter plate	1 plate (96 wells)	Rabbit anti bovine S-100 β
2.	S-100 β Standard	Lyophilized	1 vial (6.3ng)	Bovine S-100 β
3.	Labeled antibody	Liquid	1 bottle (11 mL)	Biotinylated rabbit anti bovine S-100 β
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)	2N H_2SO_4
8.	Buffer solution	Liquid	1 bottle (30 mL)	Phosphate buffer
9.	Washing solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
10.	Adhesive sheet		4 pieces	

Ⅳ. Method

<Equipment required>

1. Photometer for microtiter plate (plate reader) , which can read the extinction 2.5 at 492 nm
2. Microtiter plate shaker
3. Washing device for microtiter plate, dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1000 mL)
7. Distilled water or deionized water

<Preparatory work>

1. Preparation of the standards:
   Reconstitute the S-100β standard (lyophilized 6.3 ng/vial) with 1mL of buffer solution, which affords 6300 pg/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution which yields 3150 pg/mL standard solution. Repeat the dilution procedure to make each of 1575, 788, 394, 197 and 98 pg/mL standard solutions. Buffer solution itself is used as 0pg/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 1000 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. Discard or aspirate the solution in the wells and wash the wells 3 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.

3. Fill 70 μL of buffer solution into all of the wells first, then introduce 30 μL each of standard solution (0, 98, 197, 394, 788, 1575, 3150, 6300 pg/mL) or samples into the wells.

4. Cover the plate with adhesive sheet and incubate it at 4℃ for 15～24 hours. (Still, shaker not need)

5. After 4ºC incubation, move the plate back to room temperature waiting 30～40 minutes and take off the adhesive sheet, 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. Pipette 100 μL of Labeled antibody into the wells.

7. Cover the plate with adhesive sheet and incubate it at room temperature (20～30℃) for 2 hours. During the incubation, the plate should be shaken with a microtiter plate shaker.

8. Take off the adhesive sheet, 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. Pipette 100 μL of SA-HRP solution into the wells.

10. Cover with the adhesive sheet and incubate the plate at room temperature (20～30℃) for 2 hours. During the incubation, the plate should be shaken with microtiter plate shaker.

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

12. Take off the adhesive sheet, 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 sheet and keep it for 20 minutes at room temperature for color reaction.

14. Add 100 μL of the stopping solution into the wells to stop color reaction.

15. Read optical absorbance of the solution in the wells at 492 nm. Calculate mean absorbance values of standard solutions and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard antigen; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from the standard curve.

Ⅴ. Notes

1. EDTA-2Na 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 just before use.

3. During storage of washing solution (concentrated) at 2-8**℃**, precipitates may be observed, however they will dissolve when diluted. Diluted washing solution is stable for 6 months if stored at 2-8℃.

4. Pipetting operations may affect the precision of the assay. Pipette standard solutions or samples into each well of plate precisely. Use clean test tubes or vessels in assay, and use new tip must be used for each standard and sample solution to avoid cross contamination.

5. When concentration of S100**β** in samples is expected to exceed 6.3 ng/mL, the sample needs to be diluted with buffer solution to a proper concentration.

6. During incubation except the case at 4**℃** incubation and color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.

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

8. Perform all the determination in duplicate

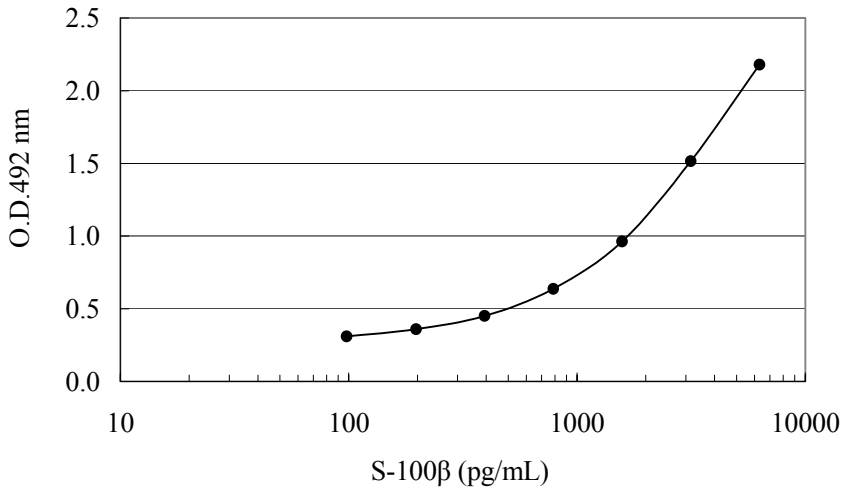
9. For accurate quantification, plot a standard curve for each assay.

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

11. 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 A>

S-100β added ng/mL	Observed ng/mL	Expected ng/mL	Recovery (%)
0.00	0.44		
0.35	0.91	0.79	115.19
1.05	1.72	1.49	115.44
3.15	3.91	3.59	108.91

<Human plasma B>

S-100β added ng/mL	Observed ng/mL	Expected ng/mL	Recovery (%)
0.00	0.26		
0.35	0.76	0.61	124.59
1.05	1.59	1.31	121.37
3.15	3.83	3.41	112.32

<Human plasma C>

S-100β added ng/mL	Observed ng/mL	Expected ng/mL	Recovery (%)
0.00	0.59		
0.35	1.05	0.94	111.70
1.05	1.88	1.64	114.63
3.15	4.34	3.74	116.04

Precision and reproducibility

- Intra-assay CV (%) 2.33 ～ 11.54
- Inter-assay CV (%) 2.91 ～ 7.77

Ⅶ. Stability and Storage

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

<Shelf life> This kit is stable under the condition for 5 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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