YK050 **Human S-100β ELISA kit**

Ⅰ. Introduction

Human S-100β 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-100β β is localized in glial cell and schwann cell, S-100α β in glial cell and S-100α α 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, severe head injury.

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of human S-100β in plasma sample.

＜Specificity＞
The ELISA kit shows 1% cross reactivity to Human S-100α α and 74% to Human S-100β β.

＜Test Principle＞
This ELISA kit for determination of human S-100β in plasma sample is based on a sandwich enzyme immunoassay. During first immune reaction, the human S-100β in standards or samples bind to the rabbit anti bovine S-100β antibodies which are coated on the surface of the microtitration plate. After rinsing out excess S-100β, Labeled antibody (Biotinylated rabbit anti bovine S-100β antibodies are added to bind to the antigen-antibody complex. Then, excess labeled antibodies are rinsed out and HRP labeled streptoavidin are added to bind to biotinylated rabbit anti bovine S-100β complex. Finally, HRP enzyme activity is determined and the concentration of human S-100β is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP*1	1 plate(96 wells)	Anti Rabbit IgG
2. S-100β Standard	Lyophilized	1 vial (6.3ng)	Bovine S-100β (6.3ng)
3. Labeled antibody	Liquid	1 bottle (11 mL)	Biotinylated Rabbit Anti bovine S-100β
4. SA-HRP solution	Liquid	1 bottle (11 mL)	HRP labeled streptoavidin
5. Buffered substrate	Liquid	1 bottle (24 mL)	0.015% Hydrogen Peroxide
6. OPD tablet	Tablet	2 tablets	o-Phenylenediamine hydrochloride
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 foil		3 sheets	

MTP*1. . . . . Microtitration plate

Ⅳ. Method

＜Equipment required＞

1）Photometer for Microtitration plate(Plate reader)、which can read the extinction 2.5 at 490 nm

2）Rotator for Microtitration plate

3）Washing device for Microtitration plate, dispenser for approximate 0.3 ml with aspiration system

4）Micropipettes, Multi-channel pipettes for 8 wells or 12 wells and their tips

5）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 standard(Lyophilized S-100β 6.3 ng/vial) with 1ml of distilled water, which makes 6300 pg/ml standard solution.. The reconstituted standard solution is to be diluted with the same volume of buffer solution (e.g. 0.2 ml standard + 0.2 ml buffer solution), that makes 3150 pg/ml standard solution. Repeat the dilution to make each standard of 1575, 788, 394, 197, 98 pg/ml. 0 pg/ml(0 pg/ml standard) is to be used with the buffer solution.

2) Preparation of the substrate solution:
Resolve the OPD tablet with the Buffered substrate.

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 and samples to room temperature before beginning the test.

2. Remove the solution from all of wells of antibody coated plate and fill 300μl/well of washing solution into the wells. Aspirate all the wells and remove any remaining washing solution.

3. Fill 70μl of Buffer solution into all of wells first, then introduce 30μl each of Standard solution (0, 98, 197, 394, 788, 3150, 6300 pg/ml) or samples into the wells.

4. Cover with adhesive foil and incubate the plate at 4℃ overnight(15 ～ 24 hours).

5. Bring the plate at room temperature (20～30℃) and stand it for 30 to 40 minutes.

6. Take off the adhesive foil, aspirate all of wells and wash four times with approximate 0.3 ml /well of washing solution.

7. Pipette 100μl of Labeled antibody into the wells.

8. Cover with adhesive foil and incubate the plate at room temperature (20～30℃) for 2 hours. During the incubation, the plate should be rotated with plate rotator.

9. Take off the adhesive foil, aspirate all of wells and wash four times with approximate 0.3 ml /well of washing solution.

10. Pipette 100μl of SA-HRP solution into the wells.

11. Cover with adhesive foil and incubate the plate at room temperature (20～30℃) for 2 hours. During the incubation, the plate should be rotated with plate rotator.

12. Take off the adhesive foil, aspirate all of wells and wash five times with approximate 0.3 ml /well of washing solution.

13. Add 100μl of the substrate solution into the wells, cover with adhesive foil and incubate the plate for 20minites at room temperature.

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

15. Read the absorbance of wells within 1 hour at 490nm..

16. Calculate the mean absorbance values of standards and plot a reference curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values.)

17. Use the reference curve to read the S-100β concentrations of samples from corresponding absorbance values.

Ⅴ. Notes on the test procedure

1. Plasma samples must be used as soon as possible after collection, if the samples are to be tested at a later time they should be devided into tubes with small amount and frozen at or below – 30℃. Avoid repeated freezing and thawing of plasma samples.

2. S-100β Standard, Buffered substrate and OPD tablet should be prepared just before their use in assay using clean test tube or vessel. However, diluted Washing solution is stable for 6 months if stored at 2 to 8℃.

3. During storage of Washing solution (Concentrated) at 2 to 8℃, precipitates may form, however they will dissolve after the dilution of the solution.

4. As pipetting operations may affect with the precision of the assay, pipette standards or samples into each well of plate exactly. And use new tips for each sample to avoid cross contamination.

5. When the sample value exceeds 6.3 ng/mL, it needs to be diluted with the buffer solution within the assay range.

6. During incubation phase except color reaction, the test plate should be rotated gently by plate rotator to promote immuno-reaction.

7. Continuous rotation of the test plate by plate rotator may cause to heat up the apparatus. It is recommended to place styrene form or plywood between the plate and the rotator.

8. Perform all the determination in duplicate.

9. Read plate (absorbance value) as soon as possible after stopping color reaction.

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

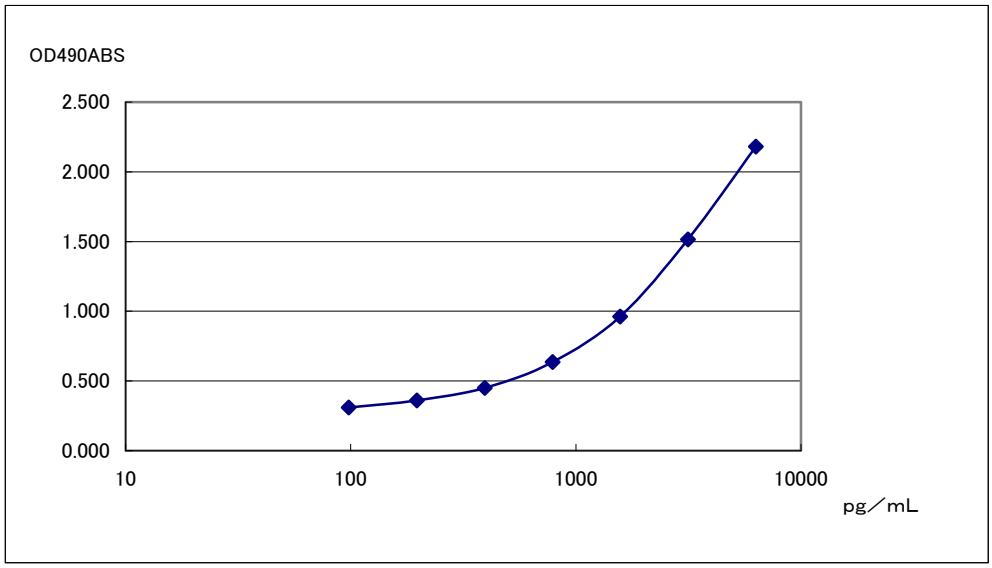
11. Protect the reagents from strong light(e.g. direct sunlight) during storage and assay.

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

Ⅵ. Performance Characteristics

Human S-100β Standard Curve

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	115.2
1.05	1.72	1.49	115.4
3.15	3.91	3.59	108.9

＜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	124.6
1.05	1.59	1.31	121.4
3.15	3.83	3.41	112.3

＜Human plasma C＞

S-100β added	Observed	Expected	Recovery
ng/mL	ng/mL	ng/mL	%
0.00	0.59		
0.35	1.05	0.94	111.7
1.05	1.88	1.64	114.6
3.15	4.34	3.74	116.0

Precision and reproducibility

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

Assay range

98 ～ 6300 pg/mL

Ⅶ. Stability and Storage

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

＜Shelf life＞　6 month from the date of manufacturing
The expiry date is described on the label of kit.

＜Package＞　For 96 tests including standards per 1 kit

Ⅷ. References

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