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SP-D KIT EIA

Research Use Only

May, 2013 (Ver. 3)

INTENDED USE

Determination of human surfactant protein D (SP-D) in serum.

KIT COMPONENTS

1. Enzyme Conjugate	0.15 mL	1 vial
2. Antibody Coated Plate		1 plate
3. Color Developing Reagent A	11 mL	1 vial
4. Color Developing Reagent B	0.5 mL	1 vial
5. Stop Solution	11 mL	1 vial
6. SP-D Standard 1 (1.56 ng/mL)	0.5 mL	1 vial
7. SP-D Standard 2 (3.13 ng/mL)	0.5 mL	1 vial
8. SP-D Standard 3 (6.25 ng/mL)	0.5 mL	1 vial
9. SP-D Standard 4 (12.5 ng/mL)	0.5 mL	1 vial
10. SP-D Standard 5 (25 ng/mL)	0.5 mL	1 vial
11. SP-D Standard 6 (50 ng/mL)	0.5 mL	1 vial
12. SP-D Standard 7 (100 ng/mL)	0.5 mL	1 vial
13. Concentrated Sample Diluent	25 mL	1 vial
14. Concentrated Washing Solution	50 mL	2 vials

ASSAY PRINCIPLE

Assay principle of this kit is based on the solid phase enzyme-linked immunosorbent assay (ELISA).

ASSAY PROCEDURE

A. Equipment

Plastic disposable tube
Tube rack
Micropipets and Multi-channel micropipet
Vortex mixer
Incubator
Aspirator or Microplate washer
Microplate reader

B. Preparation of reagents (for 1 plate)

1. Washing Solution
   Add distilled water to 50mL of Concentrated Washing Solution to the final volume of 500mL.
2. Sample Diluent
   Add distilled water to 25mL of Concentrated Sample Diluent to the final volume of 100mL.
3. Conjugate Solution
   Add 100µL of Enzyme Conjugate to 11mL of prepared Sample Diluent.
4. Substrate Mixture
   Add 50µL of Color Developing Reagent B to 11mL of Color Developing Reagent A.
   Prepare this solution just before use.

C. Preparation of samples

Use the serum as specimen
Dilute the serum to 11 times volume with Sample Diluent.
(e.g. Add 25µL of serum to 250µL of Sample Diluent)
If the SP-D level of the sample exceeds 100 ng/mL, dilute the sample with the sample diluent to obtain a value within the measuring range.

D. Standard procedure for the assay

Samples should be determined in duplicate.
Make a work sheet with Standard Solutions and samples as shown in Fig.2.
Draw the standard curve individually for each plate.

1) First incubation:
Add 100µL of Standards (1.56 to 100 ng/mL), Sample Diluent (as Standard of 0 ng/mL), and diluted samples to each well. Incubate the plate at 18-28 ºC for 2 hours.

2) Washing:
Remove the mixture from each well. Add 250µL of Washing Solution to each well. Remove the Washing Solution from each well. Further repeat the above steps twice (total 3 times).
Washing may also be done on a plate washer.
Be careful not to dry wells. Immediately add the Conjugate Solution to the wells to avoid dryness.

3) Second incubation:
Add 100 µL of Conjugate Solution to each well.
Incubate the plate at 18-28 ºC for 2 hours.

4) Washing:
Follow the same procedure in step 2).

5) Color Development:
Add 100 µL of Substrate Mixture to each well. Incubate the plate at 18-28 ºC for 30 min.
Add 100µL of Stop Solution to each well.

6) Absorbance Measurements:
Measure the absorbance at 450 nm on each well.

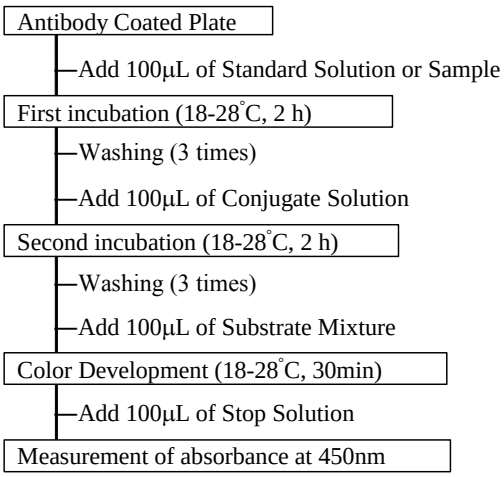

Fig.1 Flow chart of the assay procedure

	1 2	3 4	5 6	7 8	9 10	11 12
A	100	Sample 1				
B	50	Sample 2				
C	25	Sample 3				
D	12.5					
E	6.25					
F	3.13					
G	1.56					
H	0					Sample 40

Fig.2 Example of work sheet

E. Calculation of result and standard curve

1) Plot the Absorbance for each SP-D standard (vertical axis) versus the concentration (horizontal axis), and make the standard-curve by drawing a best-fit line through the points (Fig. 3).
2) Calculate SP-D levels of unknown samples by the interpolation from the standard curve.

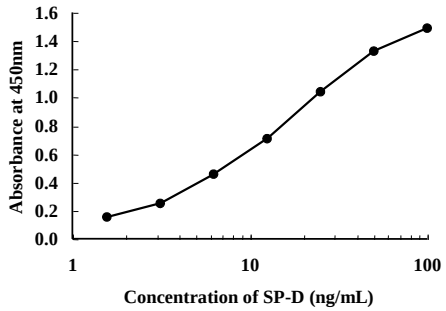

Fig. 3 Standard curve of SP-D

EFFECT OF INTERFERING SUBSTANCES

The performance of this kit is not affected by the following blood components:
Albumin (≤30 g/dL), Hemoglobin (≤2.5 g/dL), Bilirubin (Free; ≤50 mg/dL), Bilirubin (conjugated; ≤220 mg/dL)
Rheumatoid factor (≤1500 IU/mL), Chyle (≤7000 FTU)

PRECAUTION FOR USE AND HANDLING

1) The assay operation shall be done in the indicated temperatures and times.
2) Prepared reagents shall be stored at 2-8 ºC, and be used within 28 days.
   The Substrate Mixture shall not be stored and be prepared just before use.
3) The Stop Solution, 0.5M sulfuric acid solution shall be handled with care.
4) The kit components do not contain Thimerosal, sodium azide and materials from human serum.

STORAGE AND STABILITY

Kit shall be stored at 2-8 ºC.
Kit is stable until expiration date shown in QC report.

REFERENCE

1) Inoue, T. et al.: J. Immunol. Methods. 173: 157-164, 1994
2) Honda, Y. et al.: Am. J. Respir. Crit. Care. Med. 152: 1860-1886, 1995
3) Nagae, H. et al.: Clin. Chim. Acta. 266: 157-161, 1997

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