EIA For Rat/Mouse Surfactant Protein D

Rat/Mouse SP-D Kit YAMASA EIA

NOTE FOR USE

1. This kit is for research use only.
2. Users are recommended to read all instructions before use.
3. The assay procedure must be followed with indicated temperature and time.

KIT COMPONENTS

1.	Antibody Coated Plate: 96 microwells plate		1 plate
2.	Rat SP-D Standard 1 (0.47 ng/mL)	0.5mL	1 vial
3.	Rat SP-D Standard 2 (1.88 ng/mL)	0.5mL	1 vial
4.	Rat SP-D Standard 3 (7.5 ng/mL)	0.5mL	1 vial
5.	Rat SP-D Standard 4 (30 ng/mL)	0.5mL	1 vial
6.	Sample Diluent	50mL	1 vial
7.	Concentrated Washing Solution	50mL	1 vial
8.	Enzyme Conjugate	0.15mL	1 vial
9.	Enzyme Conjugate Diluent	15mL	1 vial
10.	Color Developing Reagent A	11mL	1 vial
11.	Color Developing Reagent B	0.5mL	1 vial
12.	Stop Solution	11mL	1 vial

PRINCIPLE OF THE ASSAY

Assay principle of this kit is the solid phase enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies against rat SP-D and rat SP-D as a standard material. Both anti rat SP-D antibodies also can react to mouse SP-D. It is possible to measure the concentration of rat and mouse SP-D in serum and/or bronchoalveolar lavage fluid (BALF) by this kit.

SAMPLE COLLECTION & PREPARATION

1. If samples are not analysed immediately, they shall be kept at -20°C until assay.
2. Samples that have been repeated freeze-thaw cycles and/or hemolyzed serums shall not be used.
3. All kit components and samples are warmed up to room temperature (18 to 27°C) before use.

ASSAY PROCEDURE

A. Preparation of Reagents

1. Enzyme Conjugate Solution
   Add 100µL of Enzyme Conjugate to 10mL of Enzyme Conjugate Diluent.
   This solution can be used for up to 28 days if stored at -30°C.
2. Substrate Mixture
   Mix Color Developing Reagent A and Color Developing Reagent B at a ratio of one hundred to one.
   Note: ***This solution should always be prepared just before use***.
3. Washing Solution
   Dilute Concentrated Washing Solution five fold with purified water.
   This solution can be used for up to 28 days if stored at 2 - 8°C.
4. Other components
   Antibody Coated Plate and other reagents in this kit are provided ready to use.

B. Additionally Material Required

- -Micropipettes (10, 100, 300 µL) with disposable plastic tip
- -Vibratory mixer
- -Microplate reader
- -Plastic test tube (avoid to use glass test tube)
- -Incubator
- -Aspirator for microplate or Microplate Washer
- -Purified water

C. Preparation of Sample

Serums are diluted 50-fold in rat and 10-fold in mouse with Sample Diluent. BALF from rat or mouse are diluted 100-fold with Sample Diluent. If the SP-D level of the sample exceed measuring range, dilute the sample to obtain a value within the range.

D. Standard Procedure for the Assay

Samples should be determined in duplicate. Make a work sheet with Rat SP-D Standard for standard and diluted samples as shown in Fig.1. Standard curve should be drawn individually for each assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ng/mL		Sample 4									
B	Rat SP-D Standard 1 (0.47 ng/mL)											
C	Rat SP-D Standard 2 (1.88 ng/mL)											
D	Rat SP-D Standard 3 (7.5 ng/mL)											
E	Rat SP-D Standard 4 (30 ng/mL)											
F	Sample 1											
G	Sample 2											
H	Sample 3											

Fig.1 Example of work sheet

1) Add 100µL of Rat SP-D Solution 1-4, Sample Diluent as 0ng/mL and diluted samples to each well.
2) Incubate the plate at 20 - 30 ºC for 2 hours.
3) Remove mixture from each well. Add 300µL of Washing Solution to each well. Remove Washing Solution from each well. Repeat the above steps twice. Turn the plate upside down on a paper towel. Then, remove any residual liquid by tapping the plate on the blotting paper towel.
   Note: ***Take care not to dry the well.***
4) Add 100µL of Enzyme Conjugate Solution to each well.
5) Incubate the plate at 20 - 30 ºC for 1 hour.
6) Repeat step 3.
7) Add 100µL of Substrate Mixture to each well.
8) Incubate the plate at 20 - 30 ºC for 30 min.
9) Add 100µL of Stop Solution to each well.
10) Measure the absorbance of each well at 450 nm with Microplate reader.

E. Calculation of Results

Calculate the mean value of the absorbance for each set of duplicate 0 ng/mL (Blank Value), Rat SP-D Standard and sample. The Standard Values (linear scale, y-axis) are plotted against the corresponding concentration of Rat SP-D Standard (logarithmic scale, x-axis). Draw a best-fit line through the points. SP-D concentration of the samples can be calculated from the standard curve. Multiply dilution factor to the concentrations.

PRECAUTION FOR USE OR HANDLING

1. The samples from rat and/or mouse should be handled with care, as all materials of animal are potentially hazardous.
2. Stop Solution contains sulfuric acid and should be handled with care.
3. Should reagents get into your eyes or mouth, immediately rinse them with water. Take medical advice if necessary.
4. Prepared reagents should be stored under the condition described on this instruction manual.
5. Reagents from different kit lot numbers should not be combined or interchanged.
6. Any expired components should not be use.

STORAGE AND STABILITY

Store all components at 2 to 8 °C. This kit is stable for 18 months under this condition from

manufacturing date. The expiry date of kit is printed on the label of outer box.

BIBLIOGRAPHY

1. Murata M. et al. : Exp Lung Res.2010 Oct ; 36(8):463-8

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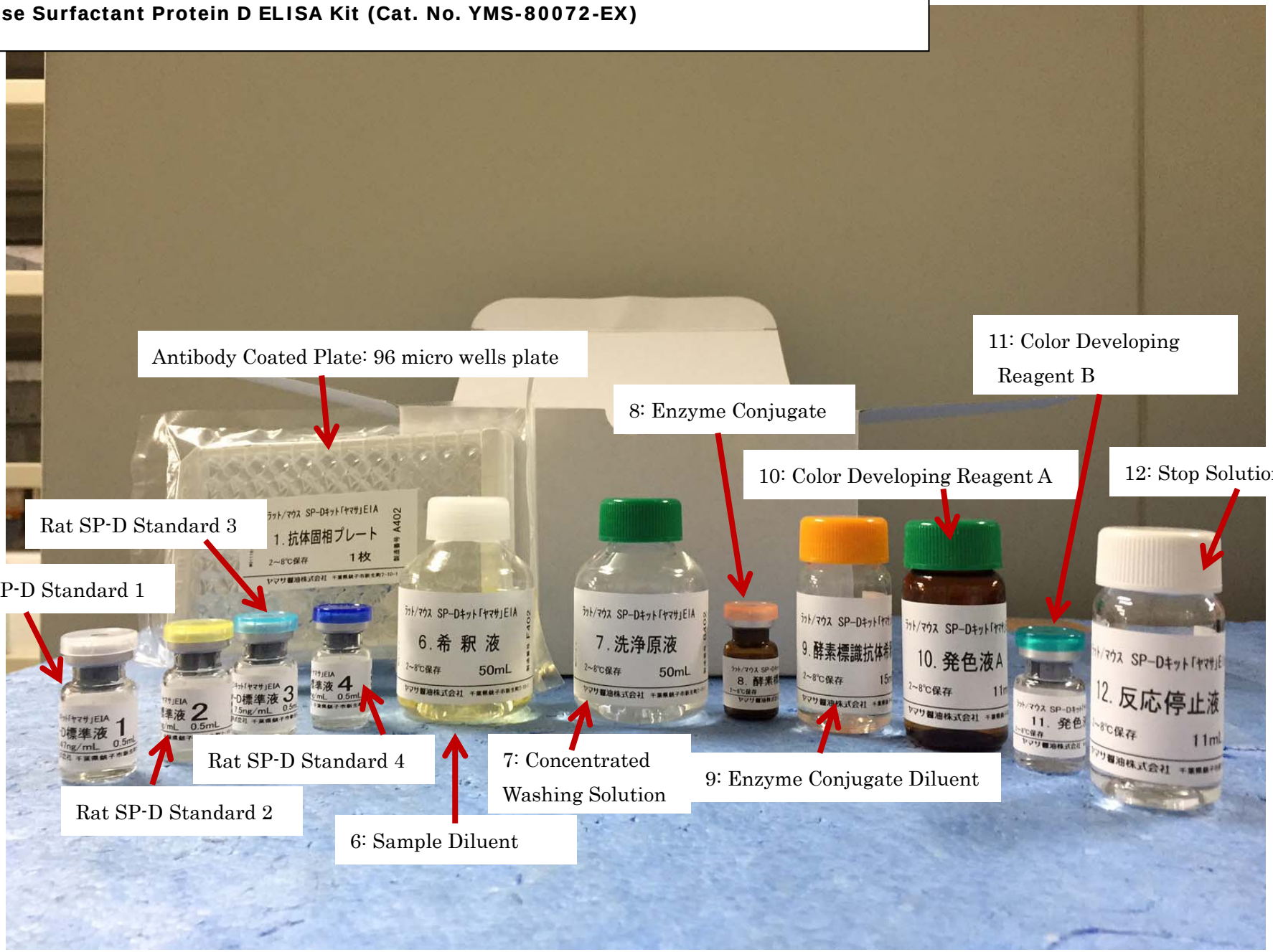
Kit Components

Rat / Mouse Surfactant Protein D ELISA Kit (Cat. No. YMS-80072-EX)