KAMIYA BIOMEDICAL COMPANY

Mouse and Rat SP-D ELISA

For the quantitative determination of SP-D in mouse and rat serum or bronchoalveolar lavage fluid

Cat. No. KT-699

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Mouse and Rat SP-D ELISA is an enzyme immunoassay for the quantitative determination of SP-D in mouse and rat serum or bronchoalveolar lavage fluid. For research use only.

INTRODUCTION

The kit for determination of SP-D in the samples obtained from rat and mouse by the enzyme immunoassay (EIA) using a microplate solid-phase method. The amounts of SP-D in the samples obtained from rat and mouse can be determined by the enzyme-substrate reaction (the chromogenic reaction) with the solid-phased antibody-SP-D-labeled antibody complex produced by reacting a solid-phased antibody on a microplate with a SP-D in the sample and a labeled antibody.

COMPONENTS

- Enzyme-labeled antibody solution, 1 x 0.15 mL
- Enzyme-labeled antibody diluent, 1 x 15 mL
- Antibody solid-phase plate, 1 plate
- Color Developing Reagent A, 1 x 11 mL
- Color Developing Reagent B, 1 x 0.5 mL
- Stop Solution, 1 x 11 mL
- Calibrator solution 1: 0.47 ng/mL, 1 x 0.5 mL
- Calibrator solution 2: 1.88 ng/mL, 1 x 0.5 mL
- Calibrator solution 3: 7.5 ng/mL, 1 x 0.5 mL
- Calibrator solution 4: 30 ng/mL, 1 x 0.5 mL
- Concentrated Sample Diluent, 1 x 50 mL
- Concentrated Washing Solution, 1 x 50 mL

PRECAUTIONS

Samples

1) The serum or bronchoalveolar lavage fluid should be used.
2) The samples should be stored at – 20℃ or lower until the determination unless the samples are immediately determined.
3) The samples should be returned to room temperature before the assay is performed.
4) Do not use the samples which are repeatedly frozen and thawed.
5) Do not use the hemolyzed samples.

PREPARATION

A. Reagent preparation

Allow the reagents to come to room temperature prior to use.

1) Enzyme-labeled antibody

Add 10 mL of enzyme-labeled antibody diluents to 100 µL of enzyme-labeled antibody solution.
The prepared enzyme-labeled antibody solution should be stored at -30℃ and used within 28 days.

2) Color Developing Reagent
JUST BEFORE USE, mix the color developing reagent A with the color developing reagent B in the ratio of 100:1 to generate the necessary volume of the prepared color developing reagent.

3) Wash Solution
Dilute the concentrated wash solution 5 times with purified water.
The prepared wash solution should be stored at 2 to 8 ℃ and used within 28 days.

4) Other reagents
Ready to use.

B. Sample preparation
1) The serum or bronchoalveolar lavage fluid (BALF) should be used as a sample.
2) The diluted samples should be used. It is desirable to use 50-fold diluted samples for the use of rat serum, 10-fold diluted samples for the use of mouse serum, and 100-fold diluted samples for the use of BALF.
3) When the measured concentration is above the limit of quantification, the sample should be appropriately diluted with the diluent and this diluted sample should be determined.

ASSAY PROCEDURE
The samples should be determined in duplicate.
1) First incubation:
Add 100 µL of the diluent (0 ng/mL), each SP-D calibrator solution or samples to each well in an antibody solid phase plate. Incubate at 20 to 30 ℃ for 2 hours.
3) Washing:
Remove the mixture from the each well, and then add 300 µL of the wash solution to the each well. Repeat the washing step 2 times.
4) Invert the plate over a paper towel and pat it to remove the residual solution (DO NOT DRY the well).
5) Second incubation:
Add 100 µL of the enzyme-labeled antibody solution to all wells. Incubate at 20 to 30 ℃ for 1 hour.
7) Repeat the steps 3) to 4).
8) Color development:
Add 100 µL of the color developing reagent to all wells. Incubate at 20 to 30 ℃ for 20 minutes.
10) Add 100 µL of the stop solution to all wells.
11) Absorbance me Measurements:
Read the absorbance at 450 nm.

CALCULATION
1) Create a calibration curve on semi-log graph paper, with each calibrator concentration on the x-axis and the absorbance on the y-axis.
2) Average the duplicate reading for samples and determine the SP-D concentrations using this calibration curve and the mean optical density of the samples. Calculate the SP-D concentrations in the samples using the obtained concentrations and dilution ratio. Detection range: 0.47 to 30 ng/mL.

STORAGE AND STABILITY
This kit is frozen under transportation. Except for Calibrator solutions, those are stored at 4℃ in dark place after the arrival and do not freeze again. Reagents of EIA are stable for 12 months under this condition.

PRECAUTIONS
1. Precaution for handling
1) Because the samples may contain the infectious material, the samples should be handled with great care as a potentially infectious material.
2) Because the stop solution in this kit contains sulfuric acid, the stop solution should be carefully handled to avoid contact with skin and so on.
3) If the reagents accidentally get into the eyes or mouth, applying appropriate first aid such as thorough washing with water and then consult a doctor if necessary.

2. Precaution for use

1) The prepared reagents should be stored by the defined method and used within the defined period
2) The reagents from different lots of this kit should not be mixed to use.
3) The kit should not be used beyond the kit expiration date.
4) The reagents should not be topped up.

3. Precaution for disposal

1) The used container should be distinguished as medical or industrial waste from other waste and disposed according to regulations for waste disposal.
2) The equipment, reagents and reagent container contacted with the samples, etc., should be considered as potentially infectious and sterilized by an autoclave, etc., or soaked in an antiseptic solution such as a 1% hypochlorous acid solution.

FOR RESEARCH USE ONLY

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