Datasheet for ABIN6975187

Surfactant Protein A1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Surfactant Protein A1 (SFTPA1)
Reactivity:	Mouse
Method Type:	Competition ELISA
Detection Range:	3.12 pg/mL - 200 pg/mL
Minimum Detection Limit:	3.12 pg/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of mouse pulmonary surfactant-associated protein A (SP-A) concentrations in serum, plasma, tissue homogenates.
Sample Type:	Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of mouse SP-A. No significant cross-reactivity or interference between mouse SP-A and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between mouse SP-A and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	0.78 pg/mL

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Product Details

Components:	• Assay plate • Standard • Biotin-antibody (100 x concentrate) • HRP-avidin (100 x concentrate) • Biotin-antibody Diluent • HRP-avidin Diluent • Sample Diluent • Wash Buffer (25 x concentrate) • TMB Substrate • Stop Solution • Adhesive Strip

Target Details

Target:	Surfactant Protein A1 (SFTPA1)
Alternative Name:	surfactant protein A1 (SFTPA1 Products)
Background:	Abbreviation: SFTPA1 Alias: AC068139.6, MGC133365, PSAP, PSPA, SFTP1, SFTPA1, pulmonary surfactant apoprotein|surfactant, pulmonary-associated protein A1|surfactant, pulmonary-associated protein A1B
UniProt:	P35242

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Sample Volume:	50 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	1. Prepare reagents, samples and standards as instructed. 2. Add 100µl standard or sample to each well. Incubate 2 hours at 37 °C. 3. Remove the liquid of each well, don´t wash. 4. Add 100 µL Biotin-antibody (1x) to each well. Incubate 1 hour at 37 °C. 5. Aspirate and wash 3 times. 6. Add 100 HRP-avidin (1x) to each well. Incubate for 1 hour at 37 °C. 7. Aspirate and wash 5 times. 8. Add 90 µl TMB Sustrate to each well. Incubate 15-30 minutes at 37 °C. Protect from light. 9. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

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Application Details

Reagent Preparation:

1. Biotin-antibody (1x) - Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Biotin-antibody + 990 µL of Biotin-antibody Diluent.
2. HRP-avidin (1x) - Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of HRP-avidin + 990 µL of HRP-avidin Diluent.
3. Wash Buffer (1x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
4. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 200 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (200 pg/mL). Sample Diluent serves as the zero standard (0 pg/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

Recommend to dilute the serum or plasma samples with Sample Diluent (1:2000) before test. The suggested 2000-fold dilution can be achieved by adding 2 µL sample to 98 µL of Sample Diluent. Complete the 2000-fold dilution by adding 6 µL of this solution to 234 µL of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

Assay Procedure:

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4 °C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times.
9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate. *Samples may require dilution. Please refer to Sample Preparation section.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For

Application Details

each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 1 minute soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

Assay Precision:	Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known concentration were tested twenty times on one plate to assess. Inter-assay Precision (Precision between assays): CV%<10% Three samples of known concentration were tested in twenty assays to assess.
Restrictions:	For Research Use only

Handling

Storage:	4 °C,-20 °C
Storage Comment:	Unopened kit: Store at 2 - 8°C. Do not use the kit beyond the expiration date. Opened kit: Coated assay plate may be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp. Standard, Biotin-antibody and HRP-avidin may be

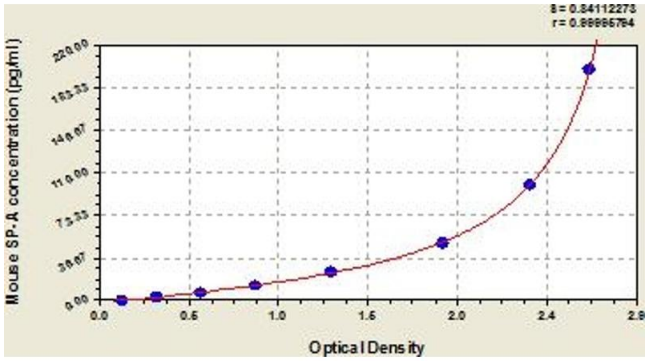
Handling

	stored for up to 1 month at 2 - 8° C. If don't make recent use, better keep it store at -20°C.Biotin-antibody Diluent, HRP-avidin Diluent, Sample Diluent, Wash Buffer, TMB Substrate and Stop Solution may be stored for up to 1 month at 2 - 8°C.
Expiry Date:	6 months

Publications

Product cited in:	Islam, Huang, Fanelli, Delsedime, Wu, Khang, Han, Grassi, Li, Xu, Luo, Wu, Liu, McKillop, Medin, Qiu, Zhong, Liu, Laffey, Li, Zhang: "Identification and Modulation of Microenvironment is Crucial for Effective MSC Therapy in Acute Lung Injury." in: **American journal of respiratory and critical care medicine**, (2018) (PubMed).

Images



ELISA

Image 1. Typical Standard Curve