Life Technologies™
Your Molecular & Cell Technology Partner

Human Pulmonary Surfactant-Associated Protein A (SP-A) ELISA Kit

to determine Human SP-A in Human Serum, Blood Plasma, Saliva, Urine,

Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT21005AKC

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INTENDED USE

This kit is used to determine Human SP-A in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only, RUO.

MANUAL VERSION 1.03

ASSAY SUMMARY

The kit is used to test the level of Human Pulmonary Surfactant-Associated Protein A (SP-A), based on the principle of double antibody sandwich technology enzyme linked immunosorbent assay (ELISA). In brief, Add Standard and Sample to the wells that are pre-coated with antibody, then add HRP-Conjugate reagent to form an immune complex, followed by incubation and washing, removal of unbound enzyme, and then addition of the substrate A and B which turns blue and finally changes into yellow under the effect of acid. The color depth or light is positively correlated with the concentration of Human SP-A.

CHARACTERISTICS

- This Kit allows for the determination of SP-A concentrations in Human serum, cell culture supernatants and other biological fluids.
- Detection range: 1pg/mL -200pg/mL.
- The minimum detectable dose (MDD) of SP-A is less than 1pg/ml.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 9%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 11%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of SP-A were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.
- Human SP-A Kit has high sensitivity and excellent specificity for detection of

SP-A. No significant cross-reactivity or interference between SP-A and analogues was observed.

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Reagents components		96T
1.	Microelisa stripplate	12*8strips
2.	Standard A	0pg/mL
3.	Standard B	12.5pg/mL
4.	Standard C	25pg/mL
5.	Standard D	50pg/mL
6.	Standard E	100pg/mL
7.	Standard F	200pg/mL
8.	Sample Diluent	6.0ml
9.	HRP-Conjugate reagent	10.0ml
10.	20X Wash solution	25ml
11.	Chromogen Solution A	6.0ml
12.	Chromogen Solution B	6.0ml
13.	Stop Solution	6.0ml
14.	Closure plate membrane	2
15.	User manual	1
16.	Sealed bags	1

Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -

80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:20.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be performed in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, and testing sample wells. Add standard 50µl to the standard wells.

3. Add Sample: Add Sample 10µl to testing sample well, then add sample diluent 40µl to testing sample well; in Blank well don't add anything.

4. Add 100µl of HRP-conjugate reagent to each well (Standard wells and Testing wells; but not in blank well), then cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.

4.Preparation of washing solution: Dilute the washing concentration (20X) with distilled or deionized water for later use.

5.Washing by hand: carefully remove the sealing film, drain the liquid by patting on absorbent paper. Fill each well with washing solution, put it aside for 1 min then drain the liquid, pat on absorbent paper, repeat 5 times. (For automatic washing: in all wells inject 350µL wash solution, soak 1min, wash plate 5 times.)

6. Add 50µl chromogen solution A to each well, then add 50µl chromogen B to

each well. Shake gently to mix up. Incubate for 15 minutes at 37°C, away from light.

7. Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution.

9.Use standards' concentrations and the corresponding OD values, to calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human SP-A concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor (e.g. it is 5 in this case).

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. 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.
6. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Stop Solution should be added to the plate in the same order as the Chromogen solution. 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 Chromogen solution.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good

laboratory practices should be followed.

9. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).