



**User's Manual**

# Human Surfactant Protein A ELISA Kit



**DEIA4795**



**96T**



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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**Creative Diagnostics**

 **Address: 45-1 Ramsey Road, Shirley, NY 11967, USA**

 **Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)**  **Fax: 1-631-938-8221**

 **Email: [info@creative-diagnostics.com](mailto:info@creative-diagnostics.com)**  **Web: [www.creative-diagnostics.com](http://www.creative-diagnostics.com)**

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## PRODUCT INFORMATION

### Intended Use

The Human Surfactant Protein A (SPA) ELISA kit is used as an analytical tool for quantitative determination of Human Surfactant Protein A, SPA in serum, plasma and cell culture supernatant.

### General Description

The ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

### Principles of Testing

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Surfactant Protein A, SPA present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin-HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Surfactant Protein A, SPA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

### Reagents And Materials Provided

1. IgG1 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Concentrated Standard, Human SPA (64ng/ml) - 0.5 ml
3. Biotinylated SPD Antibody - 1 ml
4. Streptavidin-HRP Conjugate - 6 ml
5. Standard Diluent - 3 ml
6. 25X Wash Buffer - 20 ml
7. Substrate A - 6 ml
8. Substrate B - 6 ml
9. Stop Solution - 6 ml

### Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Deionized (DI) water
4. Wash bottle or automated microplate washer



5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

## Storage

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

## Specimen Collection And Preparation

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

**Note:** Grossly hemolyzed samples are not suitable for use in this assay.

## Reagent Preparation

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **20 ml of 25X Wash Buffer in 480 ml of DI water**.
4. **Standards Preparation:** Dilute 120 µl of original **Standard (64ng/ml)** with 120 ul of standard diluent to



generate a **64ng/ml Standard stock solution**. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
32ng/ml	Standard No.5	120 ul Standard Provided (64ng/ml) + 120 ul Standard Diluent
16ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
8ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
4ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
2ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent

## Assay Procedure

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Add **50 ul Standard** to standard well. *Note: Do not add **Biotinylated SPA Antibody** to standard well because the Standard Solution contains the biotinylated antibody.*
3. Add **40 ul Sample** to respective sample wells.
4. Pipette **10 ul Biotinylated SPA Antibody** to respective sample wells.
5. Pipette **50 ul Streptavidin-HRP Conjugate** to respective sample wells and also the standard wells. *Note: Do not add the **Streptavidin-HRP Conjugate** to the blank well.*
6. Mix well. Cover the plate with a sealer and incubate for **60 minutes** at **37°C**.
7. Aspirate and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
8. Pipette **50 ul Substrate A** followed by **50 ul Substrate B** in all the wells.
9. Incubate the plate at at 37°C for 10 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette **50 ul of Stop Solution** in all wells. The wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

## Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

## Calculation

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human Surfactant Protein A, SPA concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Human Surfactant Protein A, SPA Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

## Performance Characteristics

This kit has been validated. Please view the details herein below

### Detection Range

2-32ng/ml

### Sensitivity

0.217ng/ml

## Precautions

1. This kit is For Research Use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept in the original shipping container.
7. Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from Human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
10. In any case GLP should be applied with all general and individual regulations to the use of this kit.

## Limitations

It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2\* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be less than 0.217ng/ml.