EAGLE BIOSCIENCES

Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA

Catalog Number: SLP31-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 2.0 (07.16.15)

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INTENDED USE

The Eagle Biosciences Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA Kit is intended for the quantitative determination of the Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) concentration in human serum. The Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

SUMMARY AND EXPLANATION

The Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit is a secreted inhibitor which protects epithelial tissues from serine proteases. It is found in various secretions including seminal plasma, cervical mucus, and bronchial secretions, and has affinity for trypsin, leukocyte elastase, and cathepsin G. Its inhibitory effect contributes to the immune response by protecting epithelial surfaces from attack by endogenous proteolytic enzymes. The SLPI (ALP) protein is also thought to have broad-spectrum antibiotic activity.

Secretory Leukocyte Peptidase Inhibitor Protein has been known by many names, including SLPI, ALP Protein, Antileukoproteinase protein, ALK1 protein, ALP protein, BLPI protein, HUSI protein, HUSI-I protein, MPI protein, WAP4 protein, WFDC4 protein, secretory leukocyte peptidase inhibitor protein, ALK 1 protein, Antileukoproteinase 1 protein, Antileukoproteinase1 protein, HUSI 1 protein, HUSI I protein, Protease inhibitor WAP4 protein, Seminal proteinase inhibitor protein, WAP four disulfide core domain protein 4 protein, WAP 4 protein, or WFDC 4

PRINCIPLE OF THE TEST

The Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact SLPI molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and samples are incubated with the solid phase antibody on the plate. Wells are then washed and incubated with a HRP conjugated anti-SLPI monoclonal antibody. Excess anti-SLPI-HRP antibody is then washed off and a solution of TMB Reagent is added and incubated resulting in the development of a blue color if SLPI is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of SLPI is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

MATERIALS PROVIDED

1.	Microtiter plate coated with Monoclonal anti-SLPI	
2.	SLPI reference standards: 6 vials (ready to use)	0.35ml
3.	Calibrators 1 and 2: 1 vial each (ready to use)	0.35ml
4.	Monoclonal anti-SLPI-HRP antibody	6ml
5.	TMB Reagent (One-Step)	11ml
6.	Stop Solution	11ml
7.	Wash Concentrate 10X	30ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. 1X PBS
3. Precision pipettes
4. Disposable pipette tips
5. ELISA ready capable of reading absorbance at 450nm
6. Absorbance paper or paper towel
7. Graph paper

STORAGE AND STABILITY

1. Store the Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit at 2 – 8°C.
2. Keep microplate sealed in a dry bag with desiccants.
3. The reagents of the Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit are stable until expiration date of the kit.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. The Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit calibrators contain human source components, which have been tested and found to have no evidence of infection with HIV, HCV, or HBV. Since no method can completely rule out the presence of blood borne diseases, these reagents should be handled as if they were potentially infectious.
2. This test kit is designed for Research Use Only.
3. Please refer to the U.S. Department of Health and Human Services (Bethesda, MD, USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.
4. The components in this Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Reagents contain Thimerosal as a preservative.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting as well as following the exact time and temperature requirements prescribed is essential. Any deviation from this may yield invalid data.
7. Follow local guidelines for disposal of all waste material.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit is for use with serum samples without additives only. Bring frozen samples to room temperature and mix thoroughly before analysis.

REAGENT PREPARATION

1. Prepare 1X Wash buffer by adding contents of the bottle (30ml, 10X) to 270ml of distilled or deionized water. Store at room temperature (18-26°C).
2. Dilute samples 1:5 in 1X PBS and mix gently.

ASSAY PROCEDURE

Bring all specimens and Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit reagents to room temperature (18-26°C) and gently mix.

1. Dispense 50µl of SLPI standards, calibrators, and specimens into appropriate wells.
2. Dispense 50µl of anti-SLPI-HRP Reporter into each well and incubate at room temperature for 2 hours with gentle agitation.
3. Remove samples by emptying the plate contents into a waste container
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X was buffer. Blot on absorbance paper or paper towels after each wash.
5. Strike the microtiter plate sharply onto absorbance paper or paper towels to remove all residual liquid droplets.
6. Dispense 100µl of TMB Reagent into each well and incubate at room temperature in the dark for 30 minutes.
7. Stop the reaction by adding 100µl of Stop Solution into each well.
8. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
9. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of standards, calibrators, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of SLPI in ng/ml from the standard curve and multiply by the dilution factor.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against SLPI concentrations shown in the X axis. This standard curve is for the purpose of illustration only and should not be used to calculate

unknowns. Each user should obtain his or her own data and standard curve in each experiment.

SLPI values (ng/ml)	Absorbance (450nm)
0	0.0740
5	0.3790
10	0.7260
20	1.3310
40	2.4795
80	3.5875

CALIBRATORS
Calibrator 1 range: 5 – 25 ng/ml
Calibrator 2 range: 40 – 70 ng/ml

EXPECTED VALUES AND SENSITIVY
Healthy women are expected to have SLPI assay values below 125 ng/ml according to a limited set of post-menopausal serum samples. The minimum detectable concentration of SLPI in this assay is estimated to be 1 ng/ml.

LIMITATIONS OF THE PROCEDURE
1. Reliable and reproducible results will be obtained when the Secretory Leukocyte Peptidase Inhibitor Protein (SLPI) ELISA assay kit procedure is carried with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.