LSBio™ Human ALB / Serum AlbuminELISA Kit

Catalog No. LS-F10430

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

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www.lsbio.com

Human Serum Albumin ELISA Kit

Catalog # LS-F10430 Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This human albumin antigen assay is intended for the quantitative determination of total albumin in human plasma, serum, urine & other biological fluids. **For research use only.**

BACKGROUND

Albumin is a water-soluble protein with considerable structural stability which makes up 60% of the total protein of plasma. It functions as a carrier of hormones, enzymes, fatty acids, metal ions, and medicinal products.

ASSAY PRINCIPLE

Human albumin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, polyclonal anti-human albumin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total albumin present in the samples, is reacted with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human albumin. The amount of color development is proportional to the concentration of albumin in the sample.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human albumin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **5X Diluent:** 1 bottle of 50ml
- **Human albumin standard:** 1 vial lyophilized
- **Anti-human albumin primary antibody:** 1 vial concentrated polyclonal antibody
- **Anti-goat horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

HRP conjugated secondary antibody must be stored at ≤ -70°C. Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard• Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **1X Diluent:** 5X Diluent may contain precipitate. Warm to re-dissolve before use. Dilute 50ml of 5X diluent concentrate with 200ml of deionized water.

- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of **deionized water** directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

Dilution table for preparation of human albumin standard:

Albumin concentration (ng/ml)	Dilutions
200	800µl Diluent + 200µl (1,000ng/ml)
100	500µl Diluent + 500µl (200ng/ml)
50	500µl Diluent + 500µl (100ng/ml)
20	600µl Diluent + 400µl (50ng/ml)
10	500µl Diluent + 500µl (20ng/ml)
5	500µl Diluent + 500µl (10ng/ml)
2	600µl Diluent + 400µl (5ng/ml)
1	500µl Diluent + 500µl (2ng/ml)
0.5	500µl Diluent + 500µl (1ng/ml)
0.2	600µl Diluent + 400µl (0.5ng/ml)
0	500µl Diluent Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl albumin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures albumin antigen in the 0.5-200 ng/ml range. Samples giving human albumin levels above 200 ng/ml should be diluted in diluent before use. A 1:4,000,000 to 1:16,000,000 dilution for normal human plasma and 1:1,000 dilution for normal human urine is suggested for best results. BSA concentrations of ≥0.1µg/ml in buffers will interfere with the assay and cause high background levels.

Primary Antibody Addition

Briefly centrifuge vial before opening. Dilute 3.5µl of primary antibody in 10ml of diluent and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

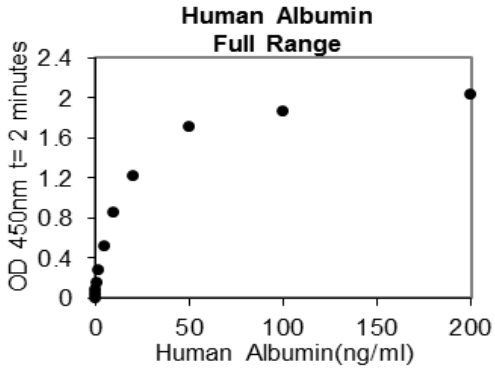
Measurement

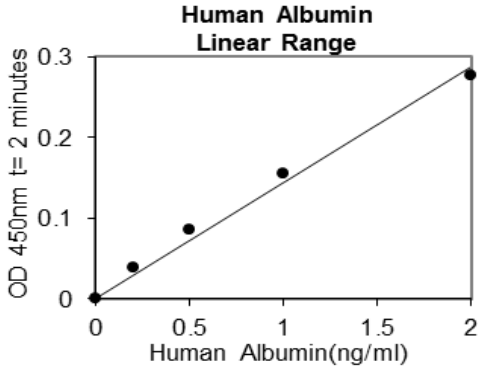
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of albumin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of albumin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

Albumin is present in human plasma and serum at a concentration of 35-50 mg/ml in adults, 30-42 mg/ml in premature infants, 35-54 mg/ml in newborns, 44-54 mg/ml in infants, and 40-59 mg/ml in children [1].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.114-0.138) and calculating the corresponding concentration. The MDD was 0.23 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	1.25	3.64	8.19
Standard Deviation	0.055	0.26	0.45
CV (%)	4.40	7.15	5.48

Inter-assay Precision: Two samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2
n	10	10
Mean (ng/ml)	4.83	10.0
Standard Deviation	0.55	1.31
CV (%)	11.3	13.1

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.73	1.51	6.74	14.1
Average % Recovery	98	101	90	94
Range	88-104%	90-109%	86-93%	92-97%

Linearity: To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	98	95	83	92
Range	82-108%	81-106%	79-9987	85-97%

Specificity: This assay recognizes natural human total albumin. Pooled normal plasma from rat, rabbit, pig, horse, sheep, and cow were assayed and no significant cross-reactivity was observed. Pooled normal plasma from mouse, guinea pig, baboon, and cyno monkey were assayed and slight cross-reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean
Citrate Plasma	1:10,000,000	34.7 mg/ml
EDTA Plasma	1:10,000,000	35.2 mg/ml
Heparin Plasma	1:10,000,000	27.6 mg/ml
Urine	1:500	7.9 µg/ml

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Pagana KD and Pagana TJ: Mosby's Diagnostic and Laboratory Test Reference, 11th Edition. 2012.

Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	
B	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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LSBio
LifeSpan BioSciences, Inc.

2401 Fourth Avenue Suite 900 Seattle, WA 98121
Tel: 206.374.1102
Fax: 206.577.4565

Technical.Support@LSBio.com