

Human Albumin (HSA) ELISA Kit

Cat. No. 1190, 96 Tests

For Quantitative Determination of Albumin
in Human Serum, plasma or culture medium

For research use only (RUO), not for diagnosis, cure or prevention of the disease.



ALPHA DIAGNOSTIC
INTERNATIONAL

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INTENDED USE

The Human Albumin (serum albumin or HSA) ELISA Kit is an immunoassay suitable for quantifying circulating serum albumin in human serum or plasma. The assay can be adapted to measure albumin in other biological fluids and solutions such as plasma, urine, and culture medium with proper control for assay compatibility. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

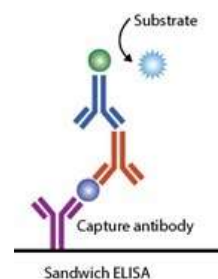
RESEARCH USE OF THE TEST

The albumins (albumen or white) are a family of globular proteins, the most common of which are the serum albumins. All the proteins of the albumin family are water-soluble. Albumins are commonly found in blood plasma and differ from other blood proteins in that they are not glycosylated. Albumin, synthesized in the liver, is the protein of the highest concentration in plasma. Albumin transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs), and is of prime importance in maintaining the osmotic pressure of the blood. Liver disease, kidney disease, and malnutrition are the major causes of low albumin. A diseased liver produces insufficient albumin. Diseased kidneys sometimes lose large amounts of albumin into the urine faster than the liver can produce it (this is termed nephritic syndrome).

Albumin (609-aa, single chain, 66.5 kda, half life 20 days) transports hormones, fatty acids, and other compounds, buffers pH, and maintains osmotic pressure, among other functions. The reference range for albumin concentrations in serum is approximately 35 - 50 g/L (3.5 - 5.0 g/dL). Plasma albumin concentration is an important indicator of nutritional status, and low concentrations pre-surgery increase the risk of post-operative wound re-opening, seroma formation and infection. Albumin levels are also dependant on the state of hydration of the body, whereby dehydration lowers albumin levels, which return to normal when the dehydration is corrected. This sensitivity to hydration state results in wide fluctuations in circulating albumin levels.

Albumin is also used in many bioprocesses and cell culture. Stem cell therapy is a promising strategy for treating many human diseases. Albumin protein expression by the liver cells or hepatocytes is used to identify proper differentiation into hepatic cells.

PRINCIPLE OF THE TEST



The Human Albumin ELISA kit is based on the binding of human albumin in samples to two antibodies, one immobilized on the microwells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of Albumin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The concentration of albumin in samples and control is calculated from a curve of standards containing known concentrations of albumin.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at RT until kit is used entirely.
Anti-Human Albumin - HRP Conjugate Concentrate (100x) Part No. 1194, 0.15ml	Peroxidase conjugated anti-Human Albumin in buffer with protein, detergents and non-azide antimicrobials as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Human Albumin Microwell Strip Plate	1191	8-well strips (12)	Coated with purified anti-human albumin antibodies.
Human Albumin Standards			
5 ng/ml	1193B	0.65 ml	Five (5) vials, each containing human serum with calibrated albumin concentrations; diluted in buffer with protein, detergents and non-azide antimicrobials as stabilizers.
10 ng/ml	1193C	0.65 ml	
20 ng/ml	1193D	0.65 ml	
50 ng/ml	1193E	0.65 ml	
100 ng/ml	1193F	0.65 ml	
Positive Control [Albumin] range on label	1192	0.65 ml	Human serum with stated albumin concentration range; diluted in buffer with protein, detergents and non-azide antimicrobials as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human Albumin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately, store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles. The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and HRP Antibody contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND, if not already on file, can be requested or obtained from the ADI website.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an albumin concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of 1:200k–1:500k are appropriate for most normal human sera. For accuracy, three dilution steps are recommended, as follows:

- 1) 10ul serum + 990ul diluent = [1:100],
- 2) 10ul [1:100] + 990ul diluent = [1:10k],
- 3) 20ul [1:10k] + 480ul diluent = [1:250k]

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human Albumin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

CALCULATION OF RESULTS

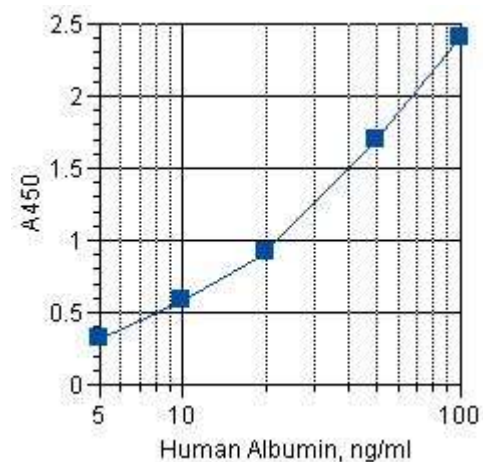
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Albumin concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Albumin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The Albumin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 100 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Alb ng/ml
A1, A2	Sample Diluent Control	0.09	0
B1, B2	5 ng/ml Standard	0.32	5
C1, C2	10 ng/ml Standard	0.59	10
D1, D2	20 ng/ml Standard	0.91	20
E1, E2	50 ng/ml Standard	1.70	50
F1, F2	100 ng/ml Standard	2.41	100
G1, G2	Positive Serum Control [Value: 24 - 46 ng/ml]	1.34	35
H1, H2	Sample [Diluted 1:800k] Calculated: 800k dilution x 44 ng/ml = 35.2 mg/ml in serum	1.58	44

A typical assay Standard Curve (do not use for calculating sample values)



PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with albumin, and have essentially no reactivity with immunoglobulins or any other human serum proteins.

Serum from rhesus monkey showed moderate reactivity in the ELISA, but monkey albumin cannot be accurately quantified in this assay. Sera from the following species showed no significant reactivity at 1:100k dilution: mouse, rat, hamster, guinea pig, bovine, pig, sheep, rabbit, goat and chicken. Fetal bovine serum at 10% also showed no reactivity.

Normal Range

Assay values of albumin in sera from 20 adult humans ranged from 14.1 to 52.4 mg/ml (median: 24.3 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of albumin were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Albumin concentrations were measured with good within-assay (6.8 to 11.4 %CV) and between-assay (3.5 to 6.4 %CV) reproducibility.

Sample	Albumin ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	32.1	6.8	3.5
Medium Sample	75.9	9.5	6.3
High Sample	113.5	11.4	6.4

Linearity of Dilution

Three individual human sera and purified albumin were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 93 to 96%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Human A	1:120k	148	17.8	90 %
	1:960k	23	22.0	
Human B	1:120k	132.5	15.9	97 %
	1:960k	15.6	15.0	
Human C	1:120k	163	19.6	100 %
	1:960k	20.5	19.7	
Purified Albumin	1:40k	168	6.72	99%
	1:160k	42.8	6.85	

PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery

High and low concentrations of human albumin were mixed into each of 3 serum samples. Observed assay values compared to expected values ranged from 108 to 114%, indicating accurate quantification of albumin in human serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High Albumin Conc'n		45.2	
+ Human A, 19.7 ng/ml	64.9	71.0	109 %
+ Human B, 19.8 ng/ml	65.0	72.9	112 %
+ Human C, 14.7 ng/ml	59.9	67.4	113 %
Low Albumin Conc'n		6.7	
+ Human A, 19.7 ng/ml	26.4	30.0	114 %
+ Human B, 19.8 ng/ml	26.5	28.7	108 %
+ Human C, 14.7 ng/ml	21.4	24.5	114 %

Related Items

1190	Human Serum Albumin ELISA Kit, 96 tests, Quantitative
1200	Human Serum Albumin (HSA) ELISA Kit, 96 tests, Quantitative
6300	Mouse Albumin ELISA Kit, 96 tests, Quantitative
6300-C1	Mouse Serum albumin control (level 1 ~ 1ug/ml) for ELISA
6400	Rat Albumin ELISA Kit, 96 tests, Quantitative
7000-10	Monkey Albumin ELISA Kit, 96 tests, Quantitative
7000-50	Dog Albumin ELISA Kit, 96 tests, Quantitative
700-1250-10	Albumin-X, Albumin (multiple species) removal kit (sufficient to remove 6-10 mg albumin or process ~200-300 ul serum; 10 mini-columns ~1.25 ml resin)
710-100-BSG	Goat Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests, Quantitative
710-110-BSR	Rabbit Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests, Quantitative
710-120-BSC	Chicken Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests, Quantitative
710-130-BSM	Mouse Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests, Quantitative
710-140-BSM	Human Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests, Quantitative
8000	Bovine Albumin (BSA) ELISA Kit, 96 tests, Quantitative