HUMAN SERUM ALBUMIN (WIDE RANGE) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ALBUMIN CONCENTRATIONS IN SERUM AND EDTA PLASMA

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN SERUM ALBUMIN (WIDE RANGE) ELISA
Catalog No.	SK00383-08
Lot No.	
Formulation	96 T
Standard Range	3.125 -400 ng/mL
Sensitivity	0.3 ng/mL
Sample Require	5~ 10 µL
Dilution Factor	1,000,000~4,000,000 (1000K~ 4000K) (**Optimal dilutions should be determined by each laboratory for each application**)
Sample Type	Serum and EDTA Plasma
Specificity	Human Albumin
Calibration	Human Albumin
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C for 1 month. See page 2-3 for detail
This kit contains sufficient materials to run 35~40 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

This Human Serum Albumin (Wide Range) ELISA Kit contains the necessary components required for the quantitative measurement of natural Human albumin from serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains Human albumin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify natural Human albumin samples.

ASSAY OVERVIEW

The Human Albumin ELISA kit is based on the binding of Human albumin in samples to two antibodies. One monoclonal antibody has been pre-coated onto a microplate, and the other polyclonal antibody. Standards and samples are pipetted into the wells and any albumin present is bound by the immobilized antibody. After a washing step, the anti rabbit antibody-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of albumin bound in the initial step. The color development is stopped and the intensity of the color is measured.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Albumin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against Human albumin.	**383-08-01**	**1 plate**
Albumin Standard – 4000 ng/vial of human albumin for calibration in a buffered protein base with preservative; lyophilized.	**383-08-02**	**1 vial**
Detection Antibody – 1.6 ml/vial, 10-fold concentrated rabbit antibody against Human albumin.	**383-08-03**	**1 vial**
Positive Control – one vial of human albumin; lyophilized.	**383-08-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µL of 100-fold concentrated Anti IgG-HRP Conjugate.	**ARIGHRP**	**1 vial**
10X Dilution Buffer Concentrate – 50 mL of 10-fold concentrate buffered protein based solution with preservative.	**WB02-10X**	**1 bottle**
Antibody Diluent Solution – 40 mL of buffered protein based solution with preservative.	**DB08A**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB02**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody

Concentrate, 10x Dilution Buffer concentrate (WB02-10X) and Antibody & HRP Diluent Solution (DB08A) should be stored at -20° C or -70° C.
Anti Rabbit IgG HRP Conjugate and TMB Substrate Solution should be stored only at 2-8 °C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes. Do not use any solutions contains bovine serum albumin in this ELISA assay.

SAMPLE COLLECTION AND STORAGE

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human serum and plasma samples may need a 1,000,000(1000K) ~ 4,000,000 (4000K)-fold dilution. A 100-fold dilution is 5 µL sample + 495 µL 1x Dilution Buffer. To make a 10,000-fold dilution is 5µL of 100-fold sample + 495 µL 1x Dilution Buffer. Finally, to make a 1,000,000(1000K)-fold dilution is 5 µL of 10,000-fold sample + 495 µL 1x Dilution Buffer. Finally, to make a 2,000,000 (2000K)-fold dilution is 120 µL of 1000K -fold sample + 120 µL 1x Dilution Buffer.
Finally, to make a 4000K-fold dilution is 80 µL of 1000K-fold sample + 240 µL 1x Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

10X Dilution Buffer Concentrate (WB02-10X):
10-fold concentrate WB02-10X cannot use directly. Must follow the dilution below:
Dilute 50 mL of Dilution Buffer Concentrate (10-fold) into 450 mL of deionized or distilled water (450 mL) to prepare 500 mL of **1x Dilution Buffer (WB02-1X)**.

Human Albumin Standard - Reconstitute the Albumin standard with 1 mL of 1x Dilution Buffer (WB02-1X). This reconstitution produces a stock solution of 4000ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of 1x Dilution Buffer into tubes #1. Pipette 375 µL of 1x Dilution Buffer into tubes #2 to #4. Pipette 250 µL of 1x Dilution Buffer into tubes #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **400 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	1x Dilution Buffer	Concentration
Stock	Powder	1 mL	4000 ng/ml
# 1	50 µl of stock	450 µl	400 ng/ml
# 2	125 µl of 1	375 µl	100 ng/ml
# 3	125 µl of 2	375 µl	25 ng/ml
# 4	125 µl of 3	375 µl	6.25 ng/ml
# 5	250 µl of 4	250 µl	3.125 ng/ml

Positive Control - Reconstitute the positive control with 2 mL of 1x Dilution Buffer to make positive control working solution.

Detection Antibody– Reconstitute the Detection Antibody with 1.6 mL of Antibody Diluent Solution (**DB08A**) to make 10-fold **concentrate detection antibody solution.** Pipette 10.8 mL of Antibody Diluent Solution (**DB08A**) into a 15 mL centrifuge tube and transfer 1.2 ml of 10-fold concentrated stock solution to prepare working solution.

Anti Rabbit IgG-HRP Conjugate – Pipette 11.88 mL of **Antibody Diluent Solution (DB08A)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **1x Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions**, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of working solution of **Anti Rabbit IgG HRP Conjugate** to each well. Cover with plate sealer. Incubate for 60 min on microplate shaker at room temperature. ***Protect from light***.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 2-3 minutes. **Protect from light.** There may be fast color development, please be prepared to add stop solution immediately.
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well using a microplate reader set to 450 nm within 5 minutes.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a 4-parameter logistic (4-PL) curve fit.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Serum Albumin	100
Human CRP	0
Human Transferrin	0
Human Fetuin A	0
Human Adiponectin	0
Human RBP-4	0

The serum samples from following species showed no significant cross-reactivity at 1:20000 dilution: mouse and rat.

TYPICAL DATA

The standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450NM
Blank	0 (0.111)
3.125	0.041
6.25	0.090
25	0.256
100	0.926
400	2.842

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl of Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl of Anti Rabbit IgG-HRP working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl of TMB Substrate Solution to each well. Incubate 2-3 min on the plate shaker at RT. **Protect from light.**
⇩
Add 100 µl of Stop Solution to each well. Read at 450nm within 5 min.

Use 5 µL of Human serum or plasma samples to prepare 1: 1000K or 4000K dilution.

		Final Dilution
5µL of Human sample	495 µL of 1x Dilution Buffer (DB08K)	100
5µL of 100-fold diluted sample solution	495 µL of 1x Dilution Buffer (DB08K)	10000
5µL of 10000-fold diluted sample solution	495 µL of 1x Dilution Buffer (DB08K)	**1000000 (1000K)**
120 µL of 1000000-fold diluted sample solution	120 µL of 1x Dilution Buffer (DB08K)	**2000000 (2000K)**
80 µL of 1000000-fold diluted sample solution	240 µL of 1x Dilution Buffer (DB08K)	**4000000 (4000K)**

Use 10 µL of Human serum or plasma samples to prepare 1: 1000K or 4000K dilution.

		Final Dilution
10 µL of Human sample	995 µL of 1x Dilution Buffer (DB08K)	100
10 µL of 100-fold diluted sample solution	995 µL of 1x Dilution Buffer (DB08K)	10000
10 µL of 10000-fold diluted sample solution	995 µL of 1x Dilution Buffer (DB08K)	**1000000 (1000K)**
120 µL of 1000000-fold (1000K) diluted sample solution	120 µL of 1x Dilution Buffer (DB08K)	**2000000 (2000K)**
80 µL of 1000000-fold (1000K) diluted sample solution	240 µL of 1x Dilution Buffer (DB08K)	**4000000 (4000K)**

Samples Test:

The research samples were diluted by 1x Dilution Buffer (WB02-1X). Its linearity and recovery was assayed by Serum Albumin (Wide Range) (Human) ELISA Kit SK00383-08.

Sample Type	Dilution Factor	Assayed (ng/mL)	Final (mg/mL)
Human Serum	1000K	43.278	43.278
Human Serum	2000K	21.407	42.813
Human Serum	4000K	11.727	46.906