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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Human serum albumin (HSA)

ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of Human serum albumin

Catalogue number LDG00031E

For Research Use or Further Manufacturing Only.

RUO

96 wells

Store at 2-8℃

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Leadgene® Human Serum Albumin (HSA) ELISA Kit

1. Introduction

HSA is the most abundant protein in blood, playing a crucial role in maintaining colloid osmotic pressure, transporting various molecules (such as fatty acids, hormones, drugs, etc.), and exerting antioxidant effects. Furthermore, during the production of biopharmaceutical products, HSA may be introduced due to its widespread applications (for example, as a stabilizer and excipient). Monitoring and controlling the residual amount of HSA is essential for ensuring the safety, efficacy, and regulatory compliance of these products. Leadgene® Human serum albumin ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of HSA level in sample solution. The Human serum albumin ELISA Kit is for research use only (RUO). Not suitable for use in diagnostic or therapeutic procedures.

2. Test principle

Human Serum Albumin (HSA) ELISA Kit is used to detect HSA in samples by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-HSA monoclonal antibody to the solid phase. HSA in the samples conjugates on solid phase and then react with the HRP conjugated mouse anti-HSA VHH antibody. Subsequent wash steps will residually unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of HSA level is accomplished by comparing the absorbance with standard curve.

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3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
Human serum albumin (HSA) ELISA plate Stripwell microplate with 96 anti-human serum albumin monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard Human serum albumin (HSA) lyophilized from buffered protein solution with preservatives	2 vials (Lyophilized form)	Refer to the vial label for reconstitution volume. Reconstitute by adding Standard reconstitution buffer to be a stock solution of 625 ng/mL. **(see procedure, section 8.(2))**
Standard reconstitution buffer Buffered protein solution with preservatives	2 vials (1.1 mL)	**Ready** for use
Standard & sample diluent buffer Buffered protein solution with preservatives	1 vial (12 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti-human serum albumin (HSA) monoclonal antibody in buffered protein solution with preservatives	1 vial (70 μL)	**Dilute** 200 x with **HRP-antibody conjugated diluent buffer** (see reagent preparation, section 5.A)
HRP-antibody conjugated diluent buffer Buffered solution with preservatives	1 vial (12 mL)	**Ready for use**
20 X wash buffer 20-fold concentrated solution of buffered surfactant with preservatives	1 vial (15 mL)	**Dilute** 20 x with distilled water (see reagent preparation, section 5.B)
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	**Ready** for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	**Ready** for use
Microplate sealing film	2 sheet	N/A

4. Materials required but not provided

(1) High quality distilled water
(2) 10 mL graduated pipettes
(3) 5 μL to 1000 μL adjustable single-channel micropipettes with disposable tips
(4) 50 μL to 300 μL adjustable multi-channel micropipettes with disposable tips
(5) Multi-channel micropipette reservoir

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(6) Disposable microcentrifuge tubes
(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) Timer
(9) Magnetic stirrer
(10) Vortex mixer
(11) Washer for microplates
(12) Incubator capable of maintaining temperature at 37±1°C
(13) Stripwell microplate spectrophotometer capable of reading at 450 nm
(14) Clean paper towels
(15) Disposable gloves
(16) Discard container for bio-medical waste

5. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

A. **Working HRP-antibody conjugate (1 X):** Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **HRP-antibody conjugated diluent buffer** and homogenize by vortex.
B. **Working wash buffer (1 X):** Dilute 1 volume of **20 X wash buffer** with 19 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Storage and expiration date of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8℃ after used, except the reconstituted standard, it must be stored at -80℃.
- Avoid multiple freeze-thaw cycles of the frozen reconstituted standard, and if stored properly at -80℃, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8℃ in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

7. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those people who have been properly trained.
- Wear suitable protective clothing and disposable gloves.

- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains H_2SO_4) contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Bring all the reagents and specimens to 15-30℃ prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Procedure

1. Evaluate the number of stripwell required to test the samples. Put the stripwells at room temperature (15-30℃) before use. The unused strips should be resealed in the bag and stored at 2-8℃. Each standard, blank, and sample should be assayed in duplicate.
2. Standard and sample preparation:

• **Standard preparation (in microcentrifuge tubes):**

- Refer to the vial label for reconstitution volume. Reconstitute the lyophilized standard with Standard reconstitution buffer to the concentration of 625 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C
- Add 400 µL Standard & sample diluent buffer to 100 µL of 625 ng/mL standard to make a 125 ng/mL standard (Tube 1).
- Adding 250 µL of Standard & sample diluent buffer to 250 µL of 125 ng/mL standard to make a 62.5 ng/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only containing Standard & sample diluent buffer.

• **Sample preparation:**

- 100 µL Sample. If the initial assay found samples contain human serum albumin (HSA) higher than the highest standard, the samples can be diluted with Standard & sample diluent buffer and then re-assay the samples.
- Add 100 µL of standards, blanks or samples into Human serum albumin (HSA) ELISA stripwell microplates (see Table 1). Cover with microplate sealing film and incubate sealed plate at 37℃ for 1 hour.

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- Remove the sealing film, aspirate the liquid from each well and then wash the plate three times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate sealed plate at 37℃ for 1 hour in the dark.
- Remove the sealing film, aspirate the liquid from each well and then wash the plate six times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of TMB into each well. Incubate for 10 minutes at room temperature in the dark.
- Add 50 µL Stop solution into each well.
- Read the absorbencies immediately at 450 nm after the Stop solution is added.

Table 1 An example of orientation of standards, blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (125 ng/mL)	Standard 1 (125 ng/mL)	Sample 1	Sample 5
B	Standard 2 (62.5 ng/mL)	Standard 2 (62.5 ng/mL)	Sample 1	Sample 5
C	Standard 3 (31.25 ng/mL)	Standard 3 (31.25 ng/mL)	Sample 2	Sample 6
D	Standard 4 (15.63 ng/mL)	Standard 4 (15.63 ng/mL)	Sample 2	Sample 6
E	Standard 5 (7.81 ng/mL)	Standard 5 (7.81 ng/mL)	Sample 3	Sample 7
F	Standard 6 (3.91 ng/mL)	Standard 6 (3.91 ng/mL)	Sample 3	Sample 7
G	Standard 7 (1.95 ng/mL)	Standard 7 (1.95 ng/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

9. Internal quality control

- The average absorbance of Blank: ≦ 0.1
- The average absorbance of highest concentration of standard (125 ng/mL): ≧ 1.0

10. Calculation of results

- The standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis) using four-parameter logistic (4-PL) curve fit.

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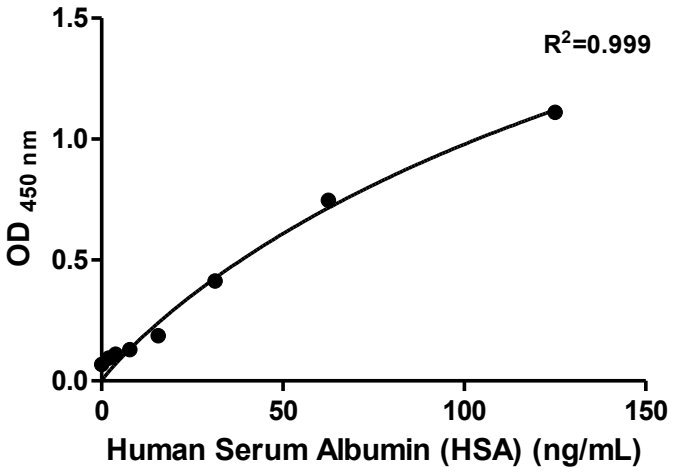
- The human serum albumin concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 1000 ng/mL, the samples should be diluted with Standard & sample diluent buffer and re-assay again.

Typical data

The following data are for demonstration only

Standard	Human serum albumin (HSA) (ng/mL)	OD 450 nm	
1	125	1.103	1.118
2	62.5	0.729	0.766
3	31.25	0.405	0.422
4	15.63	0.2	0.174
5	7.81	0.148	0.141
6	3.91	0.11	0.112
7	1.95	0.098	0.09
Blank	0	0.067	0.07



11. Assay limitations

- Sample should be centrifuged to remove debris.

12. Performance characteristics

Sensitivity

- The limit of detection (LoD) of this Human Serum Albumin (HSA) ELISA Kit is 0.486 ng/mL.
- The limit of quantification (LoQ) of this Human Serum Albumin (HSA) ELISA Kit is 1.622 ng/mL.

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