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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Interleukin-8 ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human interleukin-8 (IL-8)

Catalogue Number 70610

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

96 wells

Store at 2-8°C

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Leadgene® Interleukin-8 ELISA Kit

1. Introduction

Interleukin-8 (IL-8) is a multifunctional chemokine produced by macrophages and several cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. IL-8 is an important protein related to inflammation, that attracts neutrophils and other immune cells to the site of infection. IL-8 also participates in neutrophil chemotactic effects, mitogenesis, leukocyte activation, and calcium homeostasis.

Leadgene® IL-8 ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of IL-8 level in human serum sample. The IL-8 ELISA kit is for research use only (RUO). Not suitable for use in diagnostic or therapeutic procedures.

2. Test principle

IL-8 ELISA kit is used to detect IL-8 in human serum and recombinant human IL-8 proteins by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-IL-8 monoclonal antibody to the solid phase. IL-8 in the serum samples conjugates on solid phase and then react with the HRP conjugated mouse anti-IL-8 monoclonal antibody. Subsequent wash steps will residual unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of IL-8 level is accomplished by comparing the absorbance with standards curve.

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3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
IL-8 ELISA plate Stripwell microplate with 96 anti-IL-8 monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard Recombinant IL-8 protein lyophilized from buffered protein solution with preservatives	1 vial (Lyophilized form)	**Refer to the vial label for reconstitution volume.** Reconstitute by adding **Standard reconstitution buffer** to make a stock solution at 2 ng/mL. (see procedure, section 10.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	1 vial (1.1 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti-IL-8 monoclonal antibody in buffered protein solution with preservatives	1 vial (65 μL)	**Dilute** 200 x with **Sample & antibody diluent buffer** (see reagent preparation, section 5.A)
Assay buffer Buffered protein solution with preservatives	1 vial (6 mL)	**Ready** for use
Sample & antibody diluent buffer Buffered protein solution with preservatives	1 vial (25 mL)	**Ready** for use
10 X wash buffer 10-fold concentrated solution of buffered surfactant with preservatives	1 vial (25 mL)	**Dilute** 10 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 sheets	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
(6) Disposable microcentrifuge tubes

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(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 **Sample & antibody diluent buffer** and homogenize by vortex.
B. **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 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℃.
- 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 -20℃.
- Avoid multiple freeze-thaw cycles of the frozen reconstituted standard, and if stored properly at -20℃, 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 persons who have been properly trained.
- Human blood derivatives exhibit the risk in transmitting hepatitis, AIDS, or other infections. Therefore, the local safety procedures should be followed when handling of reagents and serum specimens.
- 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. Specimen collection, processing & storage

Human serum is suitable for use in this assay.

- **Serum sampling:**

(1) Collect whole blood by using pyrogen/endotoxin/anticoagulant free collecting tubes.
(2) Incubate the collected blood for approximately 30 minutes for complete coagulation.
(3) After clotting, centrifuge the specimens at approximately 1000 x g for 10 minutes and carefully transfer serum into microcentrifuge tubes.

- **Storage:**

(1) If analysis will be performed within 3 days after collection, serum could be stored at 2-8℃.
(2) For prolong storage, serum should be aliquoted (250-500 μl) to avoid repeated freeze-thaw cycles and stored frozen at -20℃ or lower temperature.

- **Recommendation:**

(1) Impurities will affect assay results. Therefore, precautions have to be taken during sampling to avoid impurities, such as blood cells, fibrinogen and fibrin clot, mixed into the serum.
(2) Serum should be separated from the clot or cells as soon as possible after coagulation.
(3) Avoid multiple freeze-thaw cycles of frozen serum.
(4) Prior to assay, the frozen serum should be brought to 15-30℃ slowly and mixed gently.
(5) Do not use grossly hemolyzed specimens.

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9. 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 2 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C.
- Add 800 µL **Sample & antibody diluent buffer** to 200 µL of 2 ng/mL standard to make a 400 pg/mL standard (Tube 1).
- Adding 200 µL of **Sample & antibody diluent buffer** to 200 µL of 400 pg/mL standard to make a 200 pg/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only containing **Sample & antibody diluent buffer**.

- **Sample preparation:**

- 5 µL human serum + 45 µL **Sample & antibody diluent buffer** (equal 10 X dilution, mix thoroughly). If the initial assay found samples contain IL-8 higher than the highest standard, the samples can be diluted with **Sample & antibody diluent buffer** and then re-assay the samples.

(3) Add 50 µL of standards, blanks or samples into IL-8 ELISA stripwell microplates (see Table 1), then add 50 µL of **Assay buffer** into each well immediately. Cover with microplate sealing film and incubate sealed plate at room temperature (15-30℃) for 2 hours.

(4) 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.

(5) Add 100 µL of **Working HRP-antibody conjugate** into each well. Cover with microplate sealing film and incubate sealed plate at room temperature (15-30℃) for 1 hour in the dark.

(6) 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.

(7) Add 100 µL of TMB into each well. Incubate for 8 minutes at room temperature (15-30℃) in the dark.

(8) Add 50 µL Stop solution into each well.

(9) Read the absorbencies immediately at 450 nm after the Stop solution is added.

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

	1	2	3	4
A	Standard 1 (400 pg/mL)	Standard 1 (400 pg/mL)	Sample 1	Sample 5
B	Standard 2 (200 pg/mL)	Standard 2 (200 pg/mL)	Sample 1	Sample 5
C	Standard 3 (100 pg/mL)	Standard 3 (100 pg/mL)	Sample 2	Sample 6
D	Standard 4 (50 pg/mL)	Standard 4 (50 pg/mL)	Sample 2	Sample 6
E	Standard 5 (25 pg/mL)	Standard 5 (25 pg/mL)	Sample 3	Sample 7
F	Standard 6 (12.5 pg/mL)	Standard 6 (12.5 pg/mL)	Sample 3	Sample 7
G	Standard 7 (6.25 pg/mL)	Standard 7 (6.25 pg/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

10. Internal quality control

- The average absorbance of Blank: ≦ 0.150.
- The average absorbance of highest concentration of standard (400 pg/mL): ≧ 2.000.

11. Calculation of results

- The linear regression of standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis).
- The IL-8 concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 400 pg/mL, the samples should be diluted with **Sample & antibody diluent buffer** and re-assay again.
- Add 5 µL samples in 45 µL Sample & antibody diluent buffer for assay. The actual concentration of sample should be multiplied by the factor (10 X).

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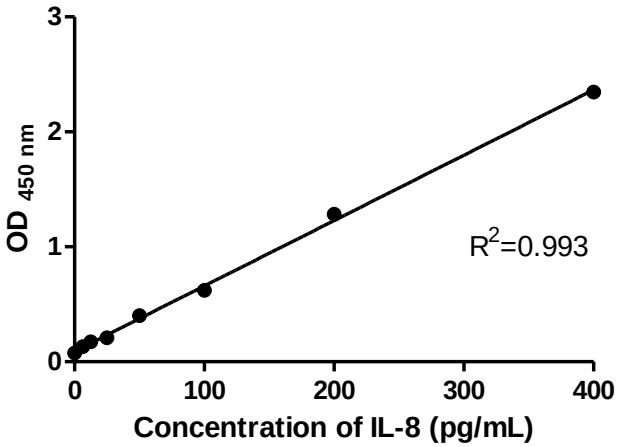
Typical data

The following data are for demonstration only

Standard	IL-8 concentration (pg/mL)	OD $_{450\ nm}$
1	400	2.345
2	200	1.285
3	100	0.621
4	50	0.402
5	25	0.208
6	12.5	0.175
7	6.25	0.132
Blank	Blank	0.075



Typical standard calibration curve for IL-8 ELISA kit

12. Assay limitations

- Sample should be centrifuged to remove debris.

13. Performance characteristics

Sensitivity

- The limit of detection (LoD) of this IL-8 ELISA kit is 5 pg/mL.
- The limit of quantification (LoQ) of this IL-8 ELISA kit is 10 pg/mL.

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