



Product Information & Manual

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Leadgene[®]

Interleukin-8 ELISA Kit

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

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

Catalogue Number: LDG00009E



96 wells

Leadgene® Interleukin-8 ELISA Kit

1. Introduction

IL-8 is a multifunctional chemokine that primarily affect the neutrophil. The phagocytic neutrophil activated by IL-8 penetrates into the inflamed tissues and produces a large number of superoxide and other cytotoxic effector molecules¹. IL-8 also promotes lysosomal activity and phagocytosis in the phagocytic neutral spheres². In addition, IL-8 plays an important role in inflammatory response by activating inflammatory cells and leading to the promotion of protein synthesis in the acute phase. In addition, the inflammatory cells release of mediators related to inflammation, thereby causing fever, inflammation and other symptoms³. In chronic inflammation, IL-8 promotes the proliferation of fibroblasts, but prolong stimulation may lead to tumor formation. In recent years, it has been found that IL-8 exhibits pleiotropic depending on cell types, such as promoting inflammation, mitosis, and angiogenesis⁴. IL-8 is considered to be highly related to immunity, multiple inflammatory diseases and tumor formation⁵. Increased levels of IL-8 have also been found in circulation of patients with schizophrenia and pregnancy-induced hypertension^{6,7}. The level of IL-8 in tumor cells is related to the outcomes of chemotherapy, and inhibition of IL-8 can improve the sensitivity to chemotherapy drugs⁸.

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.

3. Reagents provided and reconstitution

Reagents (Store at 2-8°C)	Quantity 1x96 well kit	Reconstitution
IL-8 ELISA plate Stripwell microplate with 96 anti-TEV Protease monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	Ready for use
Standard Human IL-8 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 be a stock solution of 2 ng/mL. (see procedure, section 9.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	1 vial (1.1 mL)	Ready for use
HRP-antibody conjugate HRP conjugated anti-Human IL-8 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)
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
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 ₂ SO 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

- (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 $37\pm1^{\circ}\text{C}$
- (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 20 X wash buffer with 19 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Storage and expiration dating of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at $2-8^{\circ}\text{C}$. 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 $2-8^{\circ}\text{C}$ after used, except the reconstituted standard, it must be stored at -20°C .
- Avoid multiple freeze-thaw cycles of the frozen reconstituted standard, and if stored properly at -20°C , it should be valid for maximum 2 weeks.
- Unused strips must be stored at $2-8^{\circ}\text{C}$ 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.
- Human blood derivatives exhibit the risk in transmitting hepatitis, AIDS, or other infections. Therefore, the local safety procedures should be followed when handling reagents and serum specimens.

- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB/ SB18000 and Stop solution/ ST18000, which contains H₂SO₄) or serum specimens contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of running and clean water.
- This product is intended for *in vitro* diagnostic use only and is not for use in therapeutic procedures.
- This product is designed for a single, one-time use only.
- This product is designed only for use with human serum. Other types of specimens have not been evaluated yet.
- 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°C 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°C.
- (2) For prolong storage, serum should be aliquoted (250-500 µL) to avoid repeated freeze-thaw cycles and stored frozen at -20°C 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°C slowly and mixed gently.

- (5) Do not use grossly hemolyzed specimens.

9. Procedure

- (1) Evaluate the number of stripwell required to test the samples (serum, standards and blanks). Put the stripwells at room temperature (15-30°C) before use. The unused strips should be resealed in the bag and stored at 2-8°C. 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 -80°C.
 - Add 400 µL Standard & Sample diluent buffer to 100 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 immediately. Cover with microplate sealing film and incubate sealed plate at room temperature (15-30°C) 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°C) 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 excessive wash buffer.
- (7) Add 100 µL of TMB into each well and incubate for 8 minutes at room temperature (15-30°C) in the dark.
- (8) Add 50 µL Stop solution into each well.
- (9) Read the absorbencies immediately at 450 nm.

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: ≤ 0.15 .
- The average absorbance of highest concentration of standard (400 pg/mL): ≥ 2 .

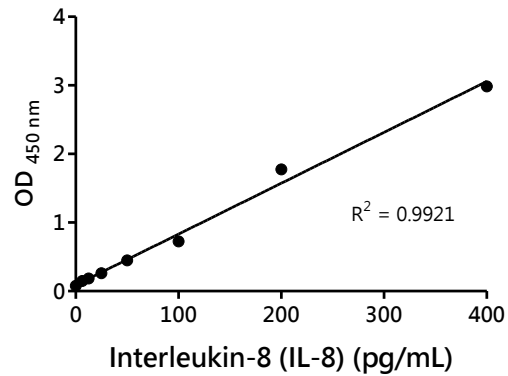
11. Calculation of results

- 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 μ L samples in 45 μ L Sample & antibody diluent buffer for assay. The actual concentration of sample should be multiplied by the factor (10 X).

Example of IL-8 standard curve

The following data are for demonstration only

Standard	IL-8 concentration (pg/mL)	OD _{450 nm}
1	400	2.985
2	200	1.776
3	100	0.725
4	50	0.448
5	25	0.262
6	12.5	0.184
7	6.25	0.148
Blank	Blank	0.078



12. Assay limitations

- Hemolysis and hyperlipidemia might affect the test result.
- Sample should be centrifuged to remove debris.

13. Performance characteristics

13.1. 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.

13.2 Specificity

- There is no significant cross reactivity in the presence of 1 µg/mL of IL-4 and IL-6.

13.3 Accuracy

Recovery rate (%)

Sample	Recovery rate (%)
Sample 1	85.2
Sample 2	86.7
Sample 3	95.1
Sample 4	92.5

- Recovery rate was evaluated by spiking of human IL-8 of four distinct concentrations in human serum samples. The range of recovery rate was from 85.2 to 95.1%.

13.4 Precision

Intra-assay

	Mean (ng/mL)	SD (ng/mL)	CV(%)
Sample 1 (n=2)	211.5	15.2	7.19
Sample 2 (n=2)	59.6	4.9	8.22
Sample 3 (n=2)	325.1	25.8	7.94
Sample 4 (n=2)	114.4	7.2	6.29

- Reproducibility within the assay was evaluated by 2 independent experiments. Each assay was carried out with 2 replicates of 4 serum samples containing different concentrations of human IL-8. The calculated intra-assay coefficient of variation was ranging from 6.29 to 8.22%.

Inter-assay

	Mean (ng/mL)	SD (ng/mL)	CV(%)
Sample 1 (n=12)	154.7	13.8	8.92
Sample 2 (n=12)	295.4	18.9	6.40
Sample 3 (n=12)	220.6	14.3	6.48
Sample 4 (n=12)	66.1	3.7	5.60

- Reproducibility within the assay was evaluated by 12 independent experiments performed by 2 technicians at different places. Each assay was carried out with 2 replicates of 4 serum samples containing different concentrations of human IL-8. The calculated inter-assay coefficient of variation was ranging from 5.60 to 8.92%.

13.5. Linearity

Range linearity %	Mean linearity %	Serial dilution up to
86 - 106	94	1:8

- Spiked human serum samples were analyzed at serial two-fold dilutions (1:1 to 1:8) with two replicates. The range of linearity was from 86 to 106% with an overall mean linearity 94%.