Interleukin-16 ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human interleukin-16 (IL-16)

Catalogue Number: LDG00006E

For Research Use or Further Manufacturing Only.

RUO

96 wells

2°C–8°C

Leadgene® Interleukin-16 ELISA Kit

1. Introduction

Interleukin-16 (IL-16), originally identified as lymphocyte chemoattractant factor (LCF), is a multifunctional immunomodulatory cytokine that primarily affects CD4-expressing immune cells. The CD4+ T cells, monocytes, and eosinophils activated by IL-16 migrate into the inflamed tissues and secrete a large number of pro-inflammatory cytokines and cytotoxic effector molecules. IL-16 also promotes the expression of IL-2 receptor (CD25) and MHC class II molecules, while modulating cell cycle progression in lymphocytes. In addition, IL-16 plays an important role in acute inflammatory and allergic responses by recruiting immune cells and stimulating the release of histamine and other inflammatory mediators, thereby causing airway hyperresponsiveness, tissue edema, and other symptoms. In chronic inflammation, prolonged stimulation of IL-16 sustains immune cell infiltration and tissue remodeling, which may contribute to autoimmune pathology and fibrosis. In recent years, it has been found that IL-16 exhibits pleiotropic functions depending on cell types and molecular forms, such as regulating T-cell activation, apoptosis, and cell proliferation. IL-16 is considered to be highly related to innate and adaptive immunity, multiple inflammatory diseases, and tumor progression. Increased levels of IL-16 have also been found in the circulation of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, asthma, and atopic dermatitis. The level of IL-16 in tumor cells and the microenvironment is related to disease progression and prognosis, and modulation of IL-16 signaling can influence anti-tumor immune responses and therapeutic outcomes.

2. Test principle

The IL-16 ELISA kit is designed for the quantitative detection of natural and recombinant human IL-16 in serum, plasma, and cell culture supernatants using the sandwich ELISA method. In this assay, a microplate is pre-coated with a mouse anti-IL-16 monoclonal capture antibody. Standards and samples are added to the wells, allowing IL-16 to bind to the immobilized antibody on the solid phase, followed by reaction with an HRP-conjugated mouse anti-IL-16 monoclonal detection antibody. After washing away unbound substances, a substrate solution is added for color development, which is then terminated by a stop solution. The optical density (OD) is measured at 450 nm, and IL-16 concentrations are determined by comparing the sample absorbance against a standard curve.

3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
IL-16 ELISA plate Stripwell microplate with 96 anti-IL-16 monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard Human IL-16 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 50 ng/mL. (see procedure, section 6.(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-16 monoclonal antibody in buffered protein solution with preservatives	1 vial (70 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 (30 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	1 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

(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 **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. Procedure

(1) Evaluate the number of stripwell required to test the samples (serum, standards and blanks). 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 50 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -80°C.
- Add 450 µL Sample & antibody diluent buffer to 50 µL of 50 ng/mL standard to make a 5,000 pg/mL standard (Tube 1).
- Adding 200 µL of Sample & antibody diluent buffer to 200 µL of 5,000 pg/mL standard to make a 2,500 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 Sample + 45 µL Sample & Antibody diluent buffer (equal 10 X dilution, mix thoroughly). If the initial assay found samples contain IL-16 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-16 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℃) 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 excessive wash buffer.

(7) Add 100 µL of TMB into each well and incubate for 10 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.

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

	1	2	3	4
A	Standard 1 (5,000 pg/mL)	Standard 1 (5,000 pg/mL)	Sample 1	Sample 5
B	Standard 2 (2,500 pg/mL)	Standard 2 (2,500 pg/mL)	Sample 1	Sample 5
C	Standard 3 (1,250 pg/mL)	Standard 3 (1,250 pg/mL)	Sample 2	Sample 6
D	Standard 4 (625 pg/mL)	Standard 4 (625 pg/mL)	Sample 2	Sample 6
E	Standard 5 (312.5 pg/mL)	Standard 5 (312.5 pg/mL)	Sample 3	Sample 7
F	Standard 6 (156.25 pg/mL)	Standard 6 (156.25 pg/mL)	Sample 3	Sample 7
G	Standard 7 (78.125 pg/mL)	Standard 7 (78.125 pg/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

7. Internal quality control

- The average absorbance of Blank: ≦ 0.15.
- The average absorbance of highest concentration of standard (5,000 pg/mL): ≧ 2.5.

8. Calculation of results

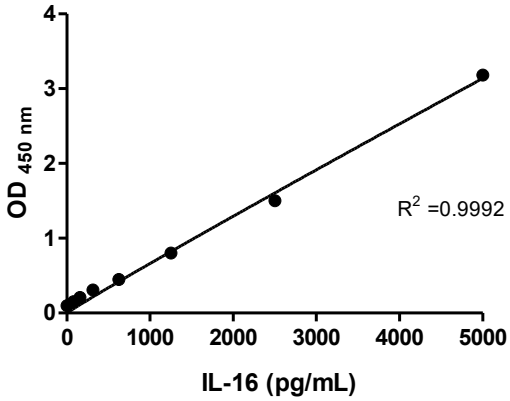
- The linear regression of standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis).
- The IL-16 concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 5,000 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).

Example of IL-16 standard curve

The following data are for demonstration only

Standard	IL-16 concentration (pg/mL)	OD $_{450\ nm}$
1	5000	3.181
2	2500	1.501
3	1250	0.801
4	625	0.448
5	312.5	0.309
6	156.25	0.207
7	78.125	0.152
Blank	Blank	0.098



9. Assay limitations

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

10. Sensitivity

- The limit of detection (LoD) of this IL-16 ELISA kit is 46.5 pg/mL.
- The limit of quantification (LoQ) of this IL-16 ELISA kit is 140.8 pg/mL.

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