

## Rat Interleukin 6 (IL6) ELISA Kit

**Catalog No.:** abx155737

**Size:** 96T

**Range:** 7.8 - 500 pg/ml

**Sensitivity:** < 3.3 pg/ml

**Storage:** Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4° C.

**Application:** For quantitative detection of IL6 in Serum, Plasma, Cell Lysates, Cell Culture Supernatants, Tissue Homegenates, and other biological fluids.

### Introduction

IL-6 is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. IL-6 is secreted by T cells and macrophages to stimulate immune response, especially after burns or other tissue damage leading to inflammation. IL-6 also plays a role in fighting infection, as IL-6 has been shown in mice to be required for resistance against bacterium *Streptococcus pneumoniae*. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10.

### Principle of the Assay

This kit is based on sandwich enzyme-linked immunosorbent assay technology. Anti-IL6 antibody is pre-coated onto 96-well plates. Biotin conjugated anti-IL6 antibody is used as detection antibody. The standards and samples are added to the wells and incubated. Next, Avidin conjugated to HRP is added. After TMB substrate solution is added only wells that contain IL6, biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the IL6 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of IL6 can be calculated.

### Kit components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Stop solution: 6 ml
10. TMB substrate : 9 ml
11. Plate sealer: 4

### Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

✧ **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.

✧ **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge at 1000 × g for 15-20 min within 30 min of collection at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C or -80°C.

✧ **Cell culture supernatant and other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. This is just an example. Rinse tissues with ice-cold PBS (0.01 mol/L, pH 7.0-7.2) to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min to collect the supernatant. Assay immediately or aliquot and store at -20°C.

✧ **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.

#### Note:

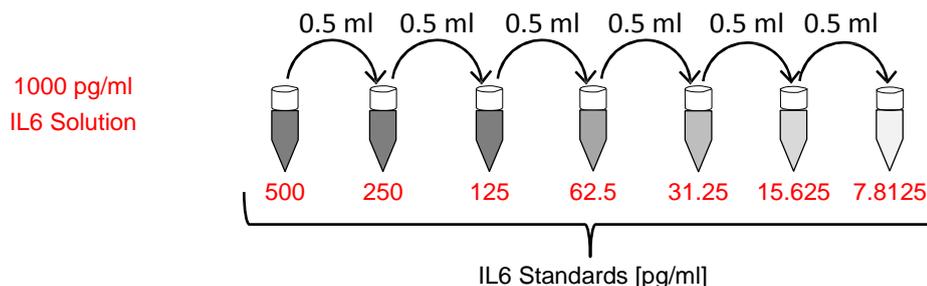
- Coagulate blood sample completely, centrifuge, and avoid hemolysis and precipitant. Please bring sample slowly to room temperature.
- Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).
- If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.
- Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

#### 2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

#### 3. Standard

Bring samples and all kit components to room temperature. Prepare the Standard with **1.0 ml** of Standard Diluent buffer to make the 1000 pg/ml Standard Solution (use within one hour), and further dilute by a factor of 2 to give the highest standard (500 pg/ml). Mix gently and avoid foaming or bubbles. Label 6 tubes with 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml and 7.8125 pg/ml respectively. Aliquot **0.5 ml** of the Standard diluent buffer into each tube. Add **0.5 ml** of 500 pg/ml standard solution into the 1<sup>st</sup> tube and mix thoroughly. Transfer **0.5 ml** from 1<sup>st</sup> tube to 2<sup>nd</sup> tube, mix thoroughly, and so on.



#### 4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B and mixed thoroughly, they are sticky solutions therefore pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 2 hours prior to the experiment. Reconstituted Standard, Detection Reagent A and B can only be used once.

#### B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100  $\mu$ l of the diluted standards into the standard wells. Aliquot 100  $\mu$ l Standard Diluent Buffer to the control (zero) well.
3. Add 100  $\mu$ l of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Seal the plate with a cover and incubate for 2 h at 37°C.
5. Remove the cover and discard the liquid of each well. Do not wash.
6. Aliquot 100  $\mu$ l of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.
7. Discard the solution and wash the plate 3 times with wash buffer. Do not let the wells completely dry at any time.

**Manual Washing:** Discard the solution without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure three times.

**Automated Washing:** Discard the solution and wash the plate three times with Wash buffer (overfilling wells with the buffer). After the final wash, invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min. Repeat this procedure three times.

8. Aliquot 100  $\mu$ l of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
9. Discard the solution and wash the plate 5 times with wash buffer (each time let the Wash Buffer stay for 1-2 min). Discard the Wash Buffer and blot the plate onto paper towels or other absorbent material.
10. Aliquot 90  $\mu$ l of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 15-25 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
11. Add 50  $\mu$ l of Stop solution into each well. The color should change to yellow immediately.

## Product Manual

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12. Read the O.D. absorbance at 450 nm in a microplate reader immediately.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The IL6 concentration of the samples can be interpolated from the standard curve.

**Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

### C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. It is recommended measuring each standard and sample in duplicate.
5. Do NOT let the wells uncovered for extended periods between incubation. Once reagents are added to the wells DO NOT let the strips dry at any time during the assay. This can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Do not reuse pipette tips and tubes to avoid cross contamination.
7. Do not use expired components or components from a different kit.
8. Store the TMB substrate in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrate for 30 min at room temperature. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

### D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level IL6 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level IL6 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%