

**IK4140 Human IL-1 $\alpha$ ELISA Kit**

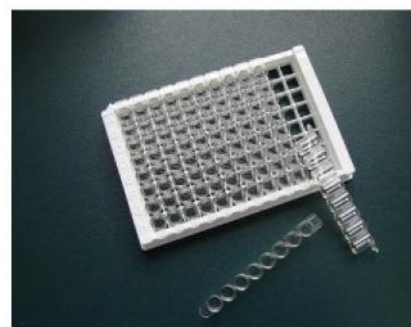
- Size:** 96 T
- Range:** 4.7 pg/ml – 300 pg/ml
- Sensitivity:** < 0.5 pg/ml
- Specificity:** No detectable cross-reactivity with any other cytokine.
- Applications:** For quantitative detection of human IL-1 $\alpha$  in cell culture supernates, serum and plasma (heparin, EDTA, Citrate)

**Principle:** Immunological Science's human IL-1 $\alpha$  ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-1 $\alpha$  specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-1 $\alpha$  amount of sample captured in plate.

**Background:** Interleukin-1 alpha (IL-1 alpha) and interleukin-1 beta (IL-1 beta) are two biochemically distinct, but distantly related, polypeptidic cytokines that play a key role in inflammation, immunologic reactions, and tissue repair. IL-1 alpha has been implicated in the pathogenesis of infectious, autoimmune and inflammatory diseases. Recently, it has been shown that IL-1 alpha is identical to hematopoietin 1, which is described as a hematopoietic growth factor acting on early progenitor cells in synergy with other hematopoietic growth factors. The human interleukin 1 alpha gene is assigned to chromosome 2. Genetic polymorphisms at interleukin (IL)-1alpha and IL-1beta have been recently suggested to be associated with severity of adult periodontitis. The murine IL-1 alpha and IL-1 beta genes encode structurally and evolutionarily related cytokines that exert a regulatory role in numerous physiological processes including hemopoiesis. The standard product used in this kit is recombinant human IL-1 $\alpha$  with the molecular mass of 18kDa.

**KIT COMPONENTS**

1. Lyophilized recombinant human IL-1 $\alpha$  standard: 10ng/tube $\times$ 2.
2. One 96-well plate precoated with anti- human IL-1 $\alpha$  antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human IL-1 $\alpha$  antibody:130ul, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130 $\mu$ l, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.



**Material Required But Not Provided**

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
  - Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  - Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6.
  - Finally, adjust the total volume to 1L.

**Notice for Application of Kit**

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

**Storage :** Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

**Expiration:** Four months at 4°C and eight months at -20°C

## Preparation

### 1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernates:** Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma:** Collect plasma using **heparin, EDTA or citrate** as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

**Note: Not suitable for serum which is haemolytic or with high fat.**

### 2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- **High target protein concentration (3-30ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (0.3-3ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (4.7-300pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤4.7pg/ml).** No dilution necessary, or the working dilution is 1:2.

### 3. Reagent Preparation and Storage

A. Reconstitution of the human IL-1α standard: IL-1α standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of IL-1α standard (10ng per tube) are included in each kit. Use one tube for each experiment.

- a. 10,000pg/ml of human IL-1α standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- b. 300pg/ml of human IL-1α standard solution: Add 0.03 ml of the above 10ng/ml IL-1α standard solution into 0.97 ml sample diluent buffer and mix thoroughly.
- c. 150pg/ml→4.7pg/ml of human IL-1α standard solutions: Label 6 Eppendorf tubes with 150pg/ml, 75pg/ml, 37.5pg/ml, 18.8pg/ml, 9.4pg/ml, 4.7pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 300pg/ml IL-1α standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

**Note:** The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human IL-1α antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than

- total volume)
- b. Biotinylated anti-human IL-1 $\alpha$  antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1  $\mu$ l Biotinylated anti-human IL-1 $\alpha$  antibody to 99  $\mu$ l antibody diluent buffer.)
- C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
- a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  - b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1  $\mu$ l ABC to 99  $\mu$ l ABC diluent buffer.)

### **Assay Procedure**

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IL-1 $\alpha$  detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-1 $\alpha$  amount in samples.

1. Aliquot 0.1ml per well of the 300pg/ml, 150pg/ml, 75pg/ml, 37.5pg/ml, 18.8pg/ml, 9.4pg/ml, 4.7pg/ml human IL-1 $\alpha$  standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum or plasma( heparin, EDTA, citrate) to each empty well. **See “Sample Dilution Guideline” above for details.** We recommend that each human IL-1 $\alpha$  standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human IL-1 $\alpha$  antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. **(Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90  $\mu$ l of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human IL-1 $\alpha$  standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative O.D.<sub>450</sub>) = (the O.D.<sub>450</sub> of each well) – (the O.D.<sub>450</sub> of Zero well). The standard curve can be plotted as the relative O.D.<sub>450</sub> of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human IL-1 $\alpha$  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.

### Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 min.
5. Add TMB stop solution and read.

### Typical Data Obtained from Human IL-1 $\alpha$

(TMB reaction incubate at 37°C for 20 min)

Concentration(pg/ml)	0.0	4.7	9.4	18.8	37.5	75	150	300
O.D	0.026	0.097	0.163	0.304	0.354	0.746	1.318	2.140

### Typical Human IL-1 $\alpha$ ELISA Kit Standard Curve

This standard curve was generated at Immunological Sciences for demonstration purpose only. A standard curve must be run with each assay.

