IMMUNOLOGICAL SCIENCES

Product Data Sheet

IK4303 Rat IL-1βELISA Kit

Size: 96 T

Range: 15.6pg/ml-1000pg/ml

Sensitivity: < 1pg/ml

Specificity: For No detectable cross-reactivity with any other cytokine.

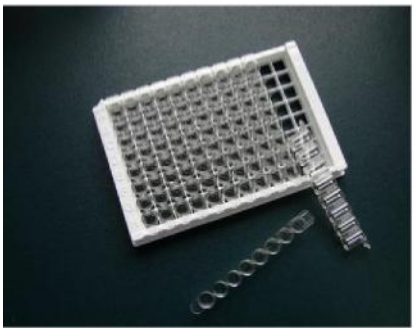
Applications: For quantitative detection of rat IL-1 β in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle: Immunological Science's rat IL-1βELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat IL-1βspecific-specific monoclonal antibodies were precoated onto 96-well plates. The rat specific detection polyclonal 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 rat IL-1βamount of sample captured in plate.

Background: The Interleukin 1 family is a group of 11 beta trefoil cytokines. Interleukin-1, produced mainly by blood monocytes, mediates the panoply of host reactions collectively known as acute phase response . Interleukin-1beta (IL-1beta) is a potent stimulator of bone resorption, and has been implicated in the pathogenesis of high bone turnover and osteoporosis. IL-1beta belongs to proinflammatory cytokines, promote cancer cell adhesion and liver metastases by up-regulating the expression of vascular cell adhesion molecule-1 (VCAM-1) on hepatic sinusoidal endothelium (HSE).

KIT COMPONENTS

1. Lyophilized recombinant rat IL-1βstandard: 10ng/tube×2.
2. One 96-well plate precoated with anti-rat IL-1βantibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti-rat IL-1βantibody : 130μl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130μl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.



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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 0.01 M **TBS:** Add 1 .2g Tris, 8.5g Nacl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H_2O 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_2HPO_4 and 0.2g NaH_2PO_4 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'tlet96-wellplatedry,dryplatewillinactivateactivecomponentsonplate.
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

Rat IL-1 β ELISA Kit

Preparation

- **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 supernate, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
  - **Serum**: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature or coat at 2-8°C overnight. Centrifuge at approximately 1500 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
  - **Plasma**: Use EDTA as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

- **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 (10-100ng/ml).** The working dilution is 1:100. i.e. Add 3 µl sample into 297 µl sample diluent buffer.
  - **Medium target protein concentration (1-10pg/ml).** The working dilution is 1:10. i.e. Add 25 µl sample into 225 µl sample diluent buffer.
  - **Low target protein concentration (15.6-1000pg/ml).** The working dilution is 1:2. i.e. Add 100 µl sample to 100 µl sample diluent buffer.
  - **Very Low target protein concentration (≤15.6pg/ml).** No dilution necessary, or the working dilution is 1:2.

- **Reagent Preparation and Storage**

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

     a. 10,000pg/ml of rat IL-1 β standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

     b. 1000pg/ml of rat IL-1 β standard solution: Add 0.1 ml of the above 10ng/ml IL-1 β standard solution into 0.9 ml sample diluent buffer and mix thoroughly.

     c. 500pg/ml → 15.6pg/ml of rat IL-1 β standard solutions: Label 6 Eppendorf tubes with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 500pg/ml IL-1 β 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 may 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-rat IL-1 β 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-rat IL-1 β antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.
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.

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 β detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-1 β amount in samples.

1. Aliquot 0.1ml per well of the 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml rat IL-1 β 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 mouse sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each rat IL-1 β 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-rat IL-1 β 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 µl 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 rat IL-1 β 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 $O.D._{450}$) = (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 mouse IL-1 β 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 Rat IL-1β

(TMB reaction incubate at 37°C for 25 min)

Concen-tration	0.0pg/ml	15.6pg/ml	31.3pg/ml	62.5pg/ml	125pg/ml	250pg/ml	500pg/ml	1000pg/ml
O.D	0.056	0.091	0.133	0.198	0.356	0.697	1.341	2.510

Typical Rat IL-1 β ELISA Kit Standard Curve

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