Rat IL-1beta ELISA Kit

Cat. No.:DEIA1174
Pkg.Size:96T

Intended use

The Rat IL-1beta ELISA kit is designed to detect and quantify the level of Rat IL1b in cell culture supernatant, serum, plasma and tissue. This kit is for research use only and not intended for diagnostic purposes.

General Description

Albumin Interleukin-1 (IL-1) was one of the first cytokines ever described. Its initial discovery was as a factor that could induce fever; control lymphocytes; increase the number of bone marrow cells and cause degeneration of bone joints. At that time; IL-1 was known under several other names including endogenous pyrogen; lymphocyte activating factor; haemopoetin-1 and mononuclear cell factor. It was around 1984-1985 when scientists confirmed that IL-1 was actually composed of two distinct proteins; now called IL-1α and IL-1β.
The original members of the IL-1 superfamily are IL-1α; IL-1β; and the IL-1 Receptor antagonist (IL-1RA). IL-1α and -β are pro-inflammatory cytokines involved in immune defense against infection. The IL-1RA is a molecule that competes for receptor binding with IL-1α and IL-1β; blocking their role in immune activation. Recent years have seen the addition of other molecules to the IL-1 superfamily including IL-18 and six more genes with structural homology to IL-1α; IL-1β or IL-1RA. These latter six members are named IL1F5; IL1F6; IL1F7; IL1F8; IL1F9; and IL1F10. In accord; IL-1α; IL-1β; and IL-1RA have been renamed IL1F1; IL1F2; and IL1F3; respectively.
Both IL-1α and IL-1β are produced by macrophages; monocytes; fibroblasts and dendritic cells. They form an important part of the inflammatory response of the body against infection. These cytokines increase the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes to sites of infection and re-set the hypothalamus thermoregulatory center; leading to an increased body temperature which expresses itself as fever. IL-1 is also important in the regulation of hematopoiesis. IL-1β production in peripheral tissue has also been associated with hyperalgesia (increased sensitivity to pain) associated with fever.
For the most part; these two forms of IL-1 bind to the same cellular receptor. This receptor is composed of two related; but non-identical; subunits that transmit intracellular signals via a pathway that is mostly shared with certain other receptors. These include the Toll family of innate immune receptors and the receptor for IL-18.

Principle Of The Test

A monoclonal antibody specific for Rat IL-1beta has been coated onto the wells of the microtiter strips provided. Standards and samples are pipetted into the wells and Rat IL-1beta binds to the immobilized antibody. After incubation, unbound substances is removed during a wash step, a biotin-conjugated anti-Rat IL-1beta antibody is added and binds to Rat IL-1beta captured by the first antibody. After incubation, unbound biotin-conjugated anti-Rat IL-1beta antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-Rat IL-1beta antibody. After incubation, unbound streptavidin-HRP is removed during a wash step, and a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of IL-1beta bound in the initial step. A colored product is formed and the reaction is terminated by the addition of stop solution. The intensity of the color is measured spectrophotometrically at 450 nm.

Reagents And Materials Provided

Rat IL-1beta Ab Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
Rat IL-1beta Standard, lyophilized recombinant Rat IL-1beta: 2 vials

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Rat IL-1beta Biotin Conjugate (biotin-labeled anti-Rat IL-1beta antibody, 100×), 30 µl per vial with preservatives: 2 vials
Streptavidin-horseradish peroxidase (HRP) Concentrate (100×), 60 µL per vial with preservatives: 2 vials
Assay Solution, 25 mL per bottle with preservatives: 2 bottles
Wash Solution Concentrate (20×), 30 mL with preservatives: 1 bottle
Chromogen Solution (Tetramethylbenzidine, TMB), 12 mL per bottle: 1 bottle
Stop Solution, 12 mL per bottle: 1 bottle

Materials Required But Not Supplied

1. A standard ELISA plate reader for absorbance at 450 nm.
2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.
7. Graduated cylinders and calibrated beakers in various sizes.

Storage

Store all reagents at 2 to 8℃.

Specimen Collection And Handling

1. The serum/plasma must be separated from the clot as early as possible as to avoid hemolysis. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters.
2. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
3. Avoid repeated freeze-thaw cycles.
4.It is recommended that all samples be diluted in Assay Solution, and the exact dilution must be determined empirically.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

A. Rat IL-1beta Standard

1. Reconstitute standard to 2000 pg/mL with 0.5 mL distilled water. Mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
2. Add 0.5 mL of Assay Solution to each of 8 tubes labeled 2000, 1000, 500, 250, 125, 62.5, 31.25 and 0 ng/mL of Rat IL-1beta.

B. Wash Solution

Allow Wash Solution Concentrate (20X) to warm up to room temperature and mix gently. Then make a 1:20 dilution of Wash Solution Concentrate (20X) with deionized or distilled water in a clean plastic tube as needed. Label as Wash Solution. Store both the concentrate and the Wash Solution in the refrigerator. The diluted buffer should be used within two weeks.

C. Biotin Conjugate Solution

Make a 1:100 dilution of the Rat IL-1beta Biotin Conjugate with Assay Solution in a clean plastic tube as needed. Label as Biotin Conjugate Solution. Biotin-labeled antibody Solution should be used within 30 minutes after dilution.

D. Streptavidin-HRP Solution

Make a 1:100 dilution of the Streptavidin-HRP Concentrate with Assay Solution in a clean plastic tube as needed. Label as Streptavidin-HRP Solution. Streptavidin-HRP Solution should be used within 30 minutes after dilution.

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Assay Steps

1. Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Dilute the stock wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the Wash buffer.
2. Diluting Sample: Dilute each sample 1:2 with sample diluent.
3. Adding Sample: Add 100µl of samples and 100µl Standard into their respective wells. Note: Use a separate disposal pipette tip for each specimen as to avoid cross-contamination.
4. Sample Incubation: Cover the plate with the plate cover and incubate for 90 minutes at 37℃. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.
5. Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remaining liquids.
6. Adding Biotin Conjugate: Add 50µl of Biotin Conjugate into each well except for the Blank.
7. Biotin Conjugate Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37℃.
8. Washing: Remove and discard the plate cover. Aspirate the liquid and rinse each well 5 times with Wash buffer (as step 5). After the final washing cycle, turn the strip plate and tap out any remainders.
9. Adding HRP Conjugate: Add 100µl of HRP-Conjugate Reagent into each well except for the Blank.
10. HRP-Conjugate Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37℃.
11. Washing: Remove and discard the plate cover. Aspirate the liquid and rinse each well 5 times with Wash buffer (as step 5). After the final washing cycle, turn the strip plate and tap out any remainders.
12. Coloring: Add 100µl of Chromogen solution into each well including the Blank. Incubate the plate at 37℃ for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-IL-1beta positive sample wells.
13. Stopping Reaction: Using a multichannel pipette or manually, add 100µl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-IL-1beta positive sample wells.
14. Measuring the Absorbance: Read the absorbance of each well at 450 nm within 10 minutes after adding the Stop Solution.

Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.
Manual Plotting: Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of Rat IL-1beta are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result in a straight line that shows a direct relationship between Rat IL-1beta concentrations and the corresponding absorbances. The concentration of Rat IL-1beta in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Rat IL-1beta concentration.
Note: Samples producing signals greater than that of the highest standard should be diluted in Assay Solution and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.
Plate Reader: An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

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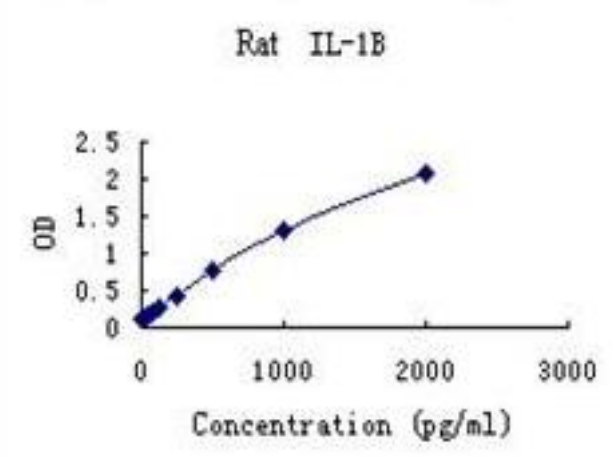

Sensitivity

The minimum detectable dose of rat IL-1beta is < 15 pg/mL.

Specificity

No detectable cross-reactivity with any other cytokine.

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<10%

Precautions

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature (18-30 ℃) before use. Shake reagent gently before, and return to 2-8 ℃ immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air -bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37℃ inside the incubator.
12. When adding samples, avoid touching the well’s bottom with the pipette tip.

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13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to rabies virus. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

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