Human IL-1 Beta ELISA Kit

Catalog Number: EA100263

Assay Principle

The OriGene Human IL1B Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human IL1B with a 96-well strip plate that is pre-coated with antibody specific for IL1B. The detection antibody is a biotinylated antibody specific for IL1B. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human IL1B with immunogen: Expression system for standard: E.coli; Immunogen sequence:A117-S269. The kit is analytically validated with ready to use reagents.

To measure Human IL1B, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human IL1B in the sample. Read the density of the yellow product in each well using a plate reader and benchmark the sample wells' readings against the standard curve to determine the concentration of Human IL1B in the sample.

Overview

Product Name	*Human IL-1 Beta ELISA Kit*
Reactive Species	*Human*
Size	*96wells/kit, with removable strips.*
Description	*Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human IL1B. 96wells/kit, with removable strips.*
Sensitivity	*<0.15 pg/ml* **The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.*
Detection Range	*3.9 pg/ml-250 pg/ml*
Storage Instructions	*Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)*
Uniprot ID	*P01584*

Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human IL-1B
Immunogen	Expression system for standard: E.coli; Immunogen sequence: A117-S-269
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.

2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.

3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.

4. Don't reuse tips and tubes to avoid cross contamination.

5. Avoid using the reagents from different batches together.

Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human IL1B Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**	**Return unused wells to the foil pouch. Reseal along the entire edge of the zip seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Human IL1B Standard	**2**	**1 ng/tube**	**Discard the IL1B stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.**
Human IL1B Biotinylated antibody (100x)	**1**	**100 μl**	**May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 μl**	
Sample Diluent	**1**	**30ml**	
Antibody Diluent	**1**	**12ml**	
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**	
Color Developing Reagent (TMB)	**1**	**10ml**	
Stop Solution	**1**	**10ml**	
Wash Buffer (25x)	**1**	**20 ml**	
Plate Sealers	**4**	**Piece**	

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 μl

through 1 ml volumes of aqueous solutions. Multichannel

pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

Test tubes for dilution.

Human IL-1 Beta ELISA Kit (EA100263) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	3.9	7.8	15.6	31.2	62.5	125	250
O.D.	0.022	0.074	0.105	0.249	0.423	0.707	1.095	1.830

Human IL-1 Beta ELISA Kit standard curve
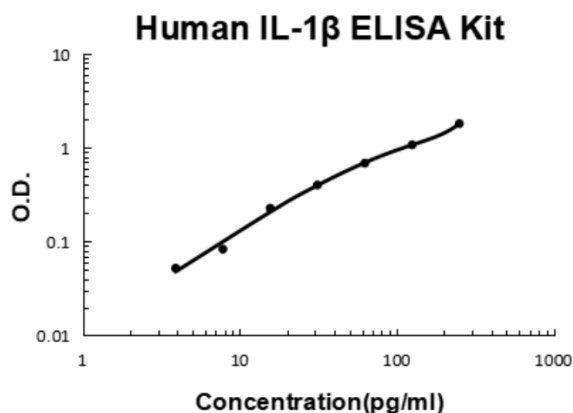
A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	11	60	123	10	58	112
Standard deviation	0.45	2.88	8.97	0.57	3.24	9.96
CV(%)	4.1	4.8	7.3	5.7	5.6	8.9

Reproducibility

To assay reproducibility, three samples with different target protein concentrations were assayed using four different lots. Number of each test n = 16.

Lots	Lot 1(pg/ml	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviations	CV (%)
Sample1	11	10	11	11	10	0.43	4.3 %
Sample2	60	54	52	53	54	3.11	5.7 %
Sample3	123	119	123	123	119	4.49	3.7 %

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-human IL1B antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human IL1B Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human IL1B Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 1 ng of lyophilized Human IL1B standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 1 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Samples	Dilute the samples so that the expected range of concentration fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for the samples.
	Internal QC testing used dilution ratio of 1:1.

Dilution of Human IL1B Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –250 pg/ml, #2 –125 pg/ml, #3 –62.50 pg/ml, #4 –31.25 pg/ml, #5 – 15.625 pg/ml, #6 – 7.81 pg/ml, #7 – 3.91 pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 250 µl of undiluted standard stock solution of 1 ng/ml and 750 µl of sample diluent to tube #1 for a final volume of 1000 µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.
It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.

4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).

5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

6. Add 100 µl of the prepared 1x Biotinylated Anti-Human IL1B antibody to each well.

7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).

8. Wash the plate 3 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.

   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).

10. Wash the plate 5 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 4 additional times.

   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)

12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.

13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic- curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on IL1B

Interleukin-1beta(IL-1beta) is a potent stimulator of bone resorption whose gene is mapped to 2q14, and has been implicated in the pathogenesis of high bone turnover and osteoporosis. IL-1beta, a prominent microglia-derived cytokine, caused oligodendrocyte death in coculture with astrocytes and microglia, but not in pure culture of oligodendrocytes alone. It also can cause nuclear export of a specific NCOR corepressor complex, resulting in derepression of a specific subset of nuclear factor-kappa-B(NFKB)-regulated genes. Furthermore, microenvironmental IL-1beta and, to a lesser extent, IL-1alpha are required for in vivo angiogenesis and invasiveness of different tumor cells. Additional, the cooperation of IL-1beta and PDGFB induces contractile-to-synthetic phenotype modulation of human aortic smooth muscle cells in culture. Moreover, the association with disease may be explained by the biologic properties of IL-1beta, which is an important proinflammatory cytokine and a powerful inhibitor of gastric acid secretion.