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ab100562
IL-1 beta Human ELISA Kit

For the quantitative measurement of human IL-1 beta in plasma and cell culture supernatants.

Δ Note: Human IL-1 beta concentration is quite low in normal plasma, it may not be detected in this assay. We have not been able to detect the endogenous human IL-1 beta in normal serum with ab100562, only in serum spiked with human IL-1 beta.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Human IL-1 beta ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human IL-1 beta in plasma and cell culture supernatants.

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This assay employs an antibody specific for human IL-1 beta coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-1 beta present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human IL-1 beta antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-1 beta bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used. Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Streptavidin solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Pre-coated IL-1 beta microplate (12 x 8 well strips)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Human IL-1 beta Standard	2 vials	-20°C
Assay Diluent A	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Detection Antibody IL-1 beta (Biotinylated anti-Human IL-1 beta)	2 vials	-20°C
300X HRP-Streptavidin concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Completely aspirate all solutions and buffers during wash steps. When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into 380 mL deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 Detection Antibody IL-1 beta (Biotinylated anti-Human IL-1 beta)

Briefly centrifuge the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 300X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP Streptavidin concentrate must be diluted 300-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 50 µL of 300X HRP-Streptavidin concentrate into a tube with 15 mL 1X Assay Diluent B to prepare a final 300-fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly centrifuge the vial of Human IL-1 beta Standard and then add 880 µL Assay Diluent A (for plasma samples) or 1X Assay Diluent B (for cell culture medium) into the Human IL-1 beta Standard vial to prepare a 20 ng/mL **Stock Standard**. Mix thoroughly but gently.

10.2 Label tubes #1-7.

10.3 Prepare the **Standard #1** by adding 5 µL **Stock Standard** into tube #1 along with 995 µL Assay Diluent A or 1x Assay Diluent B. Mix thoroughly but gently.

10.4 Add 300 µL Assay Diluent A or 1x Assay Diluent B into the remaining tubes.

10.5 Prepare **Standard #2** by adding 200 µL Standard #1 to tube #2. Mix thoroughly but gently.

10.6 Prepare **Standard #3** by adding 200 µL from Standard #2 to tube #3. Mix thoroughly but gently.

10.7 Using the table below as a guide, prepare further serial dilutions.

10.8 Standard #8 contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	5 µL	995	20,000	100
2	200 µL Standard #1	300	100	40
3	200 µL Standard #2	300	40	16
4	200 µL Standard #3	300	16	6.4
5	200 µL Standard #4	300	6.4	2.56
6	200 µL Standard #5	300	2.56	1.02
7	200 µL Standard #6	300	1.02	0.48
8	-	300	0	0

11. Sample Preparation

General Sample Information:

- If your samples need to be diluted, Assay Diluent A should be used for dilution of plasma samples. 1X Assay Diluent B should be used for dilution of culture supernatants.
- Suggested dilution for normal plasma: 2-fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Add 100 µL of each standard (see standard preparations, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over-night at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µL) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3 Add 100 µL of 1X Biotinylated IL-1 beta Detection Antibody (Reagent Preparation, section 9.3) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9.4) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

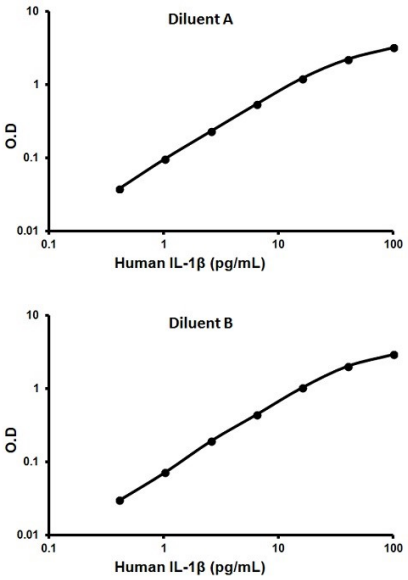

Figure 1. Example of typical human IL-1 beta standard curve using Assay Diluent A or B. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of IL-1 beta is typically less than 0.3 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of human IL-1 beta into human plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Plasma	99.66	90-107
Cell culture media	100.43	89-110

LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value	Range (%)
1:2	99	89-107
1:4	95	88-105

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	98	88-105
1:4	97	91-107

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

17. Assay Specificity

The antibodies used within this ELISA kit detect human IL-1 beta.

Cross Reactivity:

This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1a, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1a, MIP-1β, MIP-1, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-a, TNF-β, TPO, VEGF).

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

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