

HUMAN IL-1 β ELISA KIT

For the quantitative determination of human IL-1 β concentrations in cell culture supernates, serum, and plasma



FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN IL-1 β ELISA
Catalog No.	SK00746-01
Lot No.	
Formulation	96 T
Standard Range	3.9-500 pg/mL
Sensitivity	4 pg/mL
Sample Volume	100 μ l
Sample Type	Serum, EDTA Plasma, Cell Culture
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human IL-1 β only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8 $^{\circ}$ C

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INTRODUCTION

Human IL-1 β immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human IL-1 β in cell culture supernates, serum, and plasma. It contains recombinant human IL-1 β and antibodies raised against this protein. It has been shown to accurately quantify recombinant human IL-1 β . Results obtained with naturally occurring IL-1 β samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human IL-1 β . Since the measurement of IL-1 β is insensitive to the addition of recombinant forms of either of the two types of soluble receptors, it is probable that this measurement detects the total amount of IL-1 β in samples, i.e., the total amount of free IL-1 β plus the amount of IL-1 β bound to soluble receptors.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IL-1 β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1 β present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for IL-1 β is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Avidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1 β bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Code	Quantity
IL-1β Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against IL-1 β .	746-01-01	1 plate
IL-1β Standard – 5000 pg/vial of recombinant human IL-1 β in a buffered protein base with preservatives; lyophilized.	746-01-02	1 vial
Detection Antibody Concentrate – 120 μ L/vial, 100-fold concentrated of biotinylated antibody against IL-1 β with preservatives; lyophilized.	746-01-03	1 vial
Positive Control – one vial of recombinant human IL-1 β , lyophilized (optional)	746-01-04	1 vial
Avidin-HRP Conjugate – 30 μ L/vial, 500-fold concentrated solution of Avidin conjugate to HRP	AVHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservatives	DB07	1 bottle
HRP Diluent Solution – 12mL of buffered protein based solution with preservatives	DB08	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution – 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Avidin-HRP Conjugate 500-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

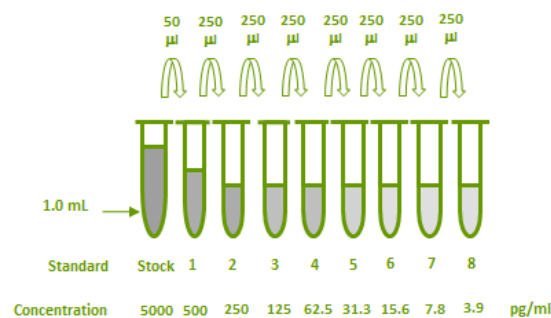
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

IL-1 β Standard - Refer to vial label for reconstitution volume. Reconstitute the IL-1 β standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipet 450 μL of Dilution Buffer into tube #1, then pipet 50 μL of stock solution into tube to produce the high standard of 500 pg/mL. Pipet 250 μL of Dilution Buffer into tubes #2 to #8. Use the high standard solution (500 pg/mL) to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Standard Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1.0 mL	5000 pg/ml
# 1	50 μL of stock	450 μL	500 pg/ml
# 2	250 μL of 1	250 μL	250 pg/ml
# 3	250 μL of 2	250 μL	125 pg/ml
# 4	250 μL of 3	250 μL	62.5 pg/ml
# 5	250 μL of 4	250 μL	31.3 pg/ml
# 6	250 μL of 5	250 μL	15.6 pg/ml
# 7	250 μL of 6	250 μL	7.8 pg/ml
# 8	250 μL of 7	250 μL	3.9 pg/ml



Positive Control - Reconstitute the positive control with 2.0 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control should be used within a few days.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 120 μ L of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipet 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 μ L of 100-fold concentrated stock solution to prepare working solution.

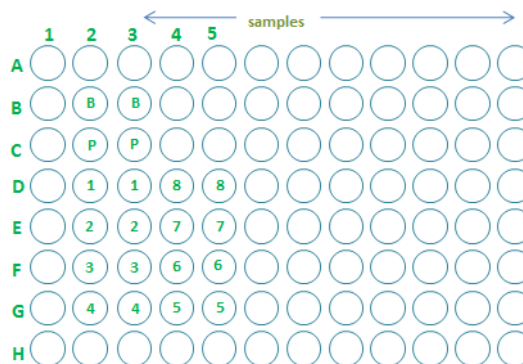
Avidin-HRP Conjugate - Pipette 11.976 mL of HRP Diluent Solution into a 15 mL centrifuge tube and transfer 24 μ L of 500-fold concentrated stock solution to prepare working solution. **Note:** 1X working solution of Avidin-HRP conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 μ L of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 μ L of Standard (D2, D3 to G2, G3 and D4, D5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 μ L) using a squirt bottle, manifold dispenser, or autowasher. 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.
6. Add 100 μ L of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of Avidin-HRP Conjugate working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**

9. Repeat the aspiration/wash as in step 5.
10. Add 100 μ L of Substrate Solution to each well. Incubate for 7-12 minutes at room temperature. **Protect from light.**
11. Add 100 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-1 β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IL-1 β .

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of IL-1 β was 4 pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.074)
3.906	0.049
7.813	0.076
15.625	0.107
31.25	0.182
62.5	0.301
125	0.566
250	0.883
500	1.585

- Lot No.:
- Positive Control: 60-125 pg/mL

SPECIFICITY

This assay recognizes both natural and recombinant human IL-1 β . The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-1 β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Cytokines:	% Cross-Reactivity
Human IL-1 α	0
Human IL-6	0
Human IL-2	0
Human IL-4	0
Human IL-10	0
Human TGF-1 β	0
Human MCP-1	0
Human MIP-1	0
Mouse IL-1 β	0
Mouse Adiponectin	0

SUMMARY OF ASSAY PROCEDURE

