ELISA Kit

Catalog #KHC0014 (96 tests)

KHC0013 (192 tests)

Human

IL-1β US

UltraSensitive

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PURPOSE

The Invitrogen Human Interleukin-1Beta UltraSensitive (Hu IL-1β US) ELISA is to be used for the quantitative determination of Hu IL-1β in human serum, EDTA plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Hu IL-1β.

INTRODUCTION

Human interleukin-1 (IL-1) is a key mediator of the host response to various infectious, inflammatory and immunologic challenges. Two distinct polypeptides, IL-1α and IL-1β, mediate IL-1 biological activities and bind to the same cell surface receptor. Both are initially synthesized as 31 kDa intracellular precursors that are subsequently found as mature proteins of 17 kDa in monocyte supernates. Membrane-bound IL-1 has also been described and may account for a part of IL-1 mediated local effects. The primary sources of IL-1 are blood monocytes and tissue macrophages. Other specialized cells such as T- and B-lymphocytes, various epithelial, endothelial and some mesenchymal cells can also produce IL-1. IL-1β is the major form secreted by monocytes and macrophages which are believed to be the main source of circulating (plasma) IL-1. Inhibitors of IL-1 activity have been described in plasma and other biological fluids. IL-1 affects several unrelated tissues and is a main mediator of the "acute phase" inflammatory responses characterized by alterations in metabolic, endocrinologic and immunologic functions. This cytokine has an essential role in T-cell activation, providing one of the necessary signals for IL-2 (T-cell growth factor) production.

It is the main mediator of inflammatory processes by acting on the nervous system, on bone marrow-derived cells (chemotaxis and/or activation of neutrophils, monocytes and lymphocytes) and on various tissues (fibroblast proliferation, resorption of cartilage and bone matrices, glial cell proliferation, stimulation of endothelial cell procoagulant activity, etc.). Most of these activities are directly attributable to IL-1β, but others are mediated in collaboration with other cytokines such as IL-6, interferons, and tumor necrosis factor. IL-1 stimulates the production or acts synergistically with these cytokines and the final biological activity is thus the result of a network of interactions between these various mediators.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use .

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Hu IL-1β US kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu IL-1β has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IL-1β content, and unknowns, are pipetted into these wells.

During the first incubation, the Hu IL-1β antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for Hu IL-1β is added. During the second incubation, this antibody binds to the immobilized Hu IL-1β captured during the first incubation.

After removal of excess secondary antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich.

After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***	***192 Test Kit***
Hu IL-1β US Standard, recombinant Hu IL-1β lyophilized. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 8 mM sodium azide, 0.05% Proclin® 300, 0.05% thymol and 0.1% benzamidin; 25 mL per bottle.	1 bottle	2 bottles
Incubation Buffer. Contains 8 mM sodium azide; 11 mL per bottle.	1 bottle	1 bottle
Hu IL-1β Antibody-Coated Wells, 96 wells per plate.	1 plate	2 plates
Hu IL-1β Biotin Conjugate (Biotin-labeled anti-Hu IL-1β). Contains 8 mM sodium azide; 11 mL per vial.	1 vial	2 vials
Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
Streptavidin-Peroxidase (HRP) Diluent. Contains 0.04% Proclin® 300; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB);* 25 mL per bottle.	1 bottle	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	4	6

Disposal Note: This kit contains materials with small quantities of sodium azide and Proclin® 300. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Proclin® 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual. (squirt bottle, manifold dispenser, etc.)
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.

4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards and samples be run in duplicate.
7. Samples that are >20 pg/mL should be diluted with *Standard Diluent Buffer* for serum/plasma samples and with corresponding medium for cell culture samples or buffered solutions.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.
15. Precipitation may appear in the *Standard Diluent Buffer* due to the presence of lipid and/or fibrin. This does not affect the performance of the assay.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Hu IL-1β Standard

The Hu IL-1β standard was calibrated against the WHO reference preparation 86/680 (NIBSC, Hertfordshire, UK EN6 3QG). One microgram equals 66,600 International Units.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 2500 pg/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.05 mL of the reconstituted standard to a tube containing 0.45 mL *Standard Diluent Buffer*. Label as 250 pg/mL Hu IL-1β. Mix.
3. Add 0.04 mL of the tube labeled 250 pg/ml to a tube containing 0.46 mL *Standard Diluent Buffer*. Label as 20 pg/mL Hu IL-1β. Mix.
4. Add 0.250 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 10, 5, 2.5, 1.25, 0.62 and 0.31 pg/mL Hu IL-1β.
5. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Hu IL-1β Standard

Standard:	Add:	Into:
20 pg/mL	Prepare as described in Step 2.	
10 pg/mL	0.250 mL of the 20 pg/mL std.	0.250 mL of the Diluent Buffer
5 pg/mL	0.250 mL of the 10 pg/mL std.	0.250 mL of the Diluent Buffer
2.5 pg/mL	0.250 mL of the 5 pg/mL std.	0.250 mL of the Diluent Buffer
1.25 pg/mL	0.250 mL of the 2.5 pg/mL std.	0.250 mL of the Diluent Buffer
0.62 pg/mL	0.250 mL of the 1.25 pg/mL std.	0.250 mL of the Diluent Buffer
0.31 pg/mL	0.250 mL of the 0.62 pg/mL std.	0.250 mL of the Diluent Buffer
0 pg/mL	0.250 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Biotin Conjugate Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)

Note: Please add the reagents in the following order:

2. Add 50 µL of the *Incubation Buffer* to zero wells and to the wells corresponding to standards, human serum, plasma, buffered solution and cell culture samples. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.

4. Add 100 µL of standards or samples in the appropriate microtiter wells. (See **REAGENT PREPARATION AND STORAGE**, Section B.). Tap gently on the side of plate to mix.
5. Cover plate with *plate cover* and incubate for **3 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
7. Pipette 100 µL of Hu IL-1β Biotin Conjugate Working Solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
8. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
10. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
11. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
13. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or**

metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

15. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
17. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
18. Read the Hu IL-1β concentrations for unknown samples from the standard curve plotted in step 17. (Samples producing signals greater than that of the highest standard (20 pg/mL) should be diluted in *Standard Diluent Buffer* for serum/plasma samples or corresponding medium for cell culture samples or buffered solutions, and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 20 pg/mL Hu IL-1β.

Standard Hu IL-1β (pg/mL)	Optical Density (450 nm)
0	0.064
	0.059
0.31	0.137
	0.139
0.62	0.221
	0.218
1.25	0.372
	0.399
2.5	0.730
	0.674
5	1.217
	1.178
10	2.150
	2.138
20	3.627
	3.519

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 20 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >20 pg/mL with *Standard Diluent Buffer* for serum/plasma samples and with corresponding medium for cell culture samples or buffered solutions; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera or plasma (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu IL-1β in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Hu IL-1β is <0.06 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times.

PRECISION

1 Intra-Assay Precision

Samples of known Human IL-1β concentration were assayed in replicates of 24 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1.0	2.3	8.1
SD	0.1	0.1	0.6
%CV	6.4	6.5	6.8

SD = Standard Deviation
CV = Coefficient of Variation

2 Inter-Assay Precision

Samples were assayed 16 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1.1	2.3	7.8
SD	0.1	0.2	0.5
%CV	7.2	7.0	6.5

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Human serum, plasma or cell culture samples containing Hu IL-1β were serially diluted over the range of the assay in *Standard Diluent Buffer* or *RPMI* containing 10% fetal bovine serum, respectively. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

	Serum			EDTA plasma			
Dilution	**Measured (pg/mL)**	**Expected (pg/mL)**	**%**	**Dilution**	**Measured (pg/mL)**	**Expected (pg/mL)**	**%**
1/1	12.3	12.3	100	1/1	19.4	19.4	100
1/2	6.2	6.2	100	1/2	10.5	9.7	108
1/4	3.1	3.1	100	1/4	5.1	4.8	106
1/8	1.57	1.54	102	1/8	2.7	2.4	112
1/16	0.88	0.77	114	1/16	1.41	1.21	116
1/32	0.40	0.38	105	1/32	0.62	0.61	102

Cell Culture			
Dilution	**Measured (pg/mL)**	**Expected (pg/mL)**	**%**
1/2	10.8	10.8	100
1/4	5.0	5.4	93
1/8	2.8	2.7	102
1/16	1.35	1.35	100
1/32	0.79	0.67	118

RECOVERY

The recovery of Hu IL-1β added to human serum or EDTA plasma averaged 91% and 95% respectively. The recovery of Hu IL-1β added to tissue culture medium containing 1% fetal bovine serum averaged 108%, while the recovery of Hu IL-1β added to tissue culture medium containing 10% fetal bovine serum averaged 103%.

SPECIFICITY

Buffered solutions of a panel of substances at 10 ng/mL were assayed with the Invitrogen Hu IL-1β US kit. The following substances were tested and found to have no cross-reactivity or interference: human IL-1α, IL-1ra, IL-1sRI, IL-1sRII, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, TNF-α, TNF-β, IFN-γ, RANTES, G-CSF, MIP-1α, MIP-β, GRO-α, mouse IL-1β, rat IL-1β.

EXPECTED VALUES

Serum/Plasma

Each laboratory must establish its own normal values. For guidance, the mean of 25 normal sera was 0.6 pg/mL (range: from undetectable to 1.1 pg/mL).

The mean of 25 normal EDTA plasma samples was 1.3 pg/mL (range: from undetectable to 3.8 pg/mL).

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human IL-1β US Assay Summary