Quantikine® ELISA

Mouse IL-1β/IL-1F2 Immunoassay

Catalog Number MLB00C
SMLB00C
PMLB00C

For the quantitative determination of mouse Interleukin 1 beta (IL-1β) concentrations in cell culture supernates, tissue lysates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The Interleukin 1 (IL-1) family of proteins consists of IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1ra). IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1). IL-1 is not produced by unstimulated cells of healthy individuals with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. However, in response to inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is seen. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (2-5).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid (aa) level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (6, 7). Cleavage of the IL-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (9-11), but evidence suggests that these factors can be secreted by non-classical pathways (12, 13). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (14). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (13, 15). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (16, 17). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (18). The two IL-1 receptor types show approximately 28% homology in their extracellular domains but differ significantly in that the type II receptor has a cytoplasmic domain of only 29 aa, whereas the type I receptor has a 213 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (19). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (20). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (21, 22). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (23, 24). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (25).

The Quantikine® Mouse IL-1β/IL-1F2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-1β in cell culture supernates, tissue lysates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-1β and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse IL-1β showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-1β has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-1β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-1β bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # MLB00C	CATALOG # SMLB00C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-1β Microplate	893829	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-1β.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IL-1β Standard	893831	1 vial	3 vials	Recombinant mouse IL-1β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.*
Mouse IL-1β Control	893832	1 vial	3 vials	Recombinant mouse IL-1β in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse IL-1β Conjugate	893830	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IL-1β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservative.	
Calibrator Diluent RD5-16	895302	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	2 vials	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

MLB00C contains sufficient materials to run ELISAs on two 96 well plates.
SMLB00C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMLB00C). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 1000 mL graduated cylinder.
- Test tubes for dilution of standards.

If using cell lysate samples, the following is also required:

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).

PRECAUTIONS

Assay Diluent RD1N contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Cells must be lysed before assaying. See Sample Values section for details.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Do not use icteric samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

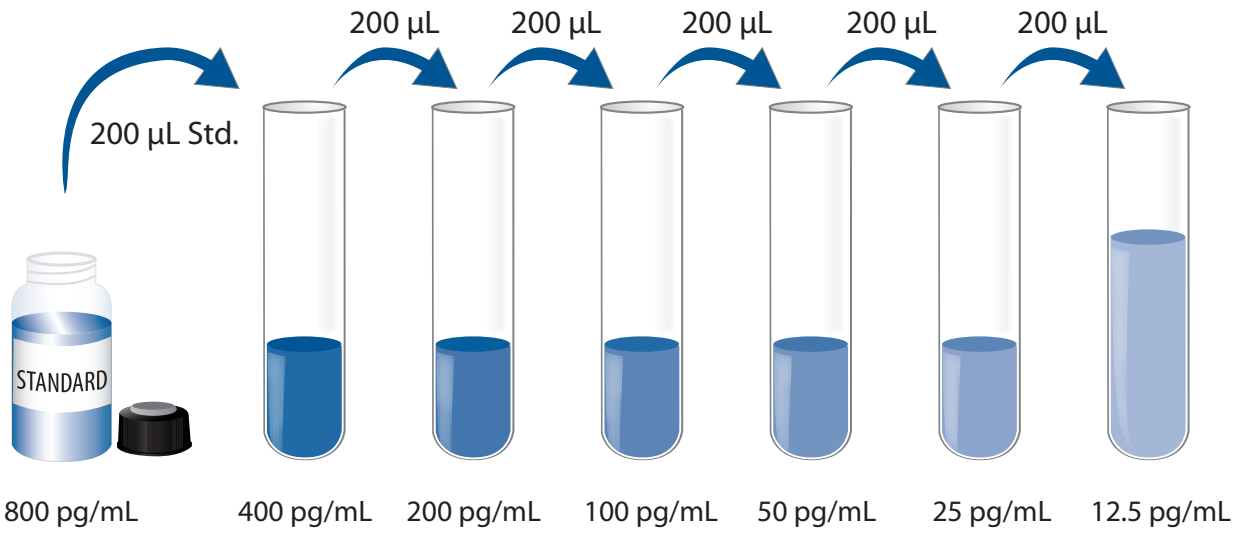
Mouse IL-1β Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse IL-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-1β Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 800 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-1β Standard (800 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents and samples as directed by the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1N to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) 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 µL of Mouse IL-1β Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

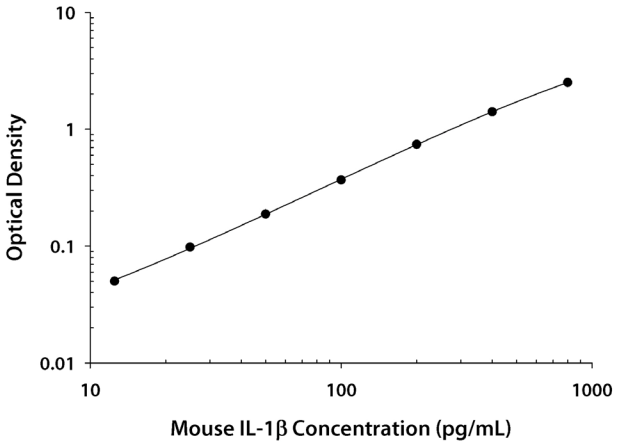
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 mouse IL-1β 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.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.040 0.042	0.041	—
12.5	0.090 0.091	0.091	0.050
25	0.133 0.144	0.139	0.098
50	0.226 0.232	0.229	0.188
100	0.408 0.410	0.409	0.368
200	0.777 0.789	0.783	0.742
400	1.422 1.476	1.449	1.408
800	2.489 2.611	2.550	2.509

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	33.5	92.9	386	34.6	93.5	423
Standard deviation	2.5	4.3	11.7	2.9	6.2	23.9
CV (%)	7.5	4.6	3.0	8.4	6.6	5.7

RECOVERY

The recovery of mouse IL-1β spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	106	95-119%
Tissue lysates (n=4)	115	110-120%
Serum (n=4)	105	98-113%
EDTA plasma (n=4)	108	101-117%
Heparin plasma (n=4)	97	87-114%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-1β were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	Tissue lysates (n=2)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	103	103	99	107
	Range (%)	94-102	100-106	99-104	98-101	98-113
1:4	Average % of Expected	94	96	107	101	108
	Range (%)	94-95	94-98	103-111	98-106	100-113
1:8	Average % of Expected	94	101	110	100	110
	Range (%)	91-96	96-105	107-113	97-103	106-115
1:16	Average % of Expected	88	96	111	98	108
	Range (%)	85-91	96-97	110-111	94-102	101-119

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of mouse IL-1β ranged from 0.46-4.80 pg/mL. The mean MDD was 2.31 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse IL-1β produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Twenty samples were evaluated for the presence of mouse IL-1β in this assay. Serum samples measured as shown in the table below. All twenty plasma samples measured less than the lowest Mouse IL-1β Standard, 12.5 pg/mL.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	27.6	10	ND-37.9

ND=Non-detectable

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells (3.5×10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum and stimulated with 5 µM β-mercapthoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was unstimulated or stimulated with 2.5 µg/mL of lipopolysacharride (LPS) and 100 ng/mL of recombinant mouse GM-CSF (R&D Systems®, Catalog # 415-ML) for 3 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse IL-1β.

Sample Type	Day 3 (pg/mL)	Day 6 (pg/mL)
J774A.1 Unstimulated	ND	ND
J774A.1 Stimulated with LPS + GM-CSF	587	938

ND=Non-detectable

Tissue Lysates - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and 1.0 mL of Lysis Buffer 2 was added (2.0 mL of Lysis Buffer 2 was added to liver tissue). Organs were lysed at room temperature for 30 minutes with gentle agitation and centrifuged to remove debris. An aliquot of each tissue lysate was removed and assayed for levels of mouse IL-1β.

Tissue Lysate	(pg/mL)
Brain	20.9
Heart	28.3
Kidney	111
Liver	347
Lung	115
Spleen	987

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-1β.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-1α
IL-1F10/IL-1HY2
IL-1ra
IL-1 RAPL2/IL-1 R9
IL-1 RI
IL-1 RII
IL-1 Rrp2/IL-1 R6
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 Rα
IL-11
IL-11 Rα
IL-12
IL-12 p40
IL-13
IL-15
IL-20
IL-23 R
ST2/IL-1 R4

Recombinant rat:

IL-1α
IL-1β

Other recombinants:

canine IL-1β
cotton rat IL-1β
human IL-1β
human pro-IL-1β
porcine IL-1β
rhesus macaque IL-1β

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