Quantikine® HS ELISA

Mouse IL-1β/IL-1F2 Immunoassay

Catalog Number MHSLB00

For the quantitative determination of mouse Interleukin 1 beta (IL-1β) concentrations in 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 cytokines consists of 11 proteins, including IL-1α and IL-1β (1). Both proteins bind to the cell surface receptor IL-1R and share many biological functions (2). IL-1β is pro-inflammatory, as protein expression dramatically increases in macrophages and monocytes in response to inflammatory agents, infections, or microbial endotoxins. In healthy individuals, it is also expressed in unstimulated skin keratinocytes, some epithelial cells, and certain cells of the central nervous system.

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-1β 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, gout, severe corticosteroid-resistant neutrophilic asthma, and aging-related diseases (3-7). Additionally, IL-1β induced neuroinflammation in the central nervous system may play a role in neuronal injury, impaired neuronal plasticity, impaired memory, and Alzheimer's disease (8-10).

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 (11, 12). Caspase-1/ICE is activated in the inflammasome and cleaves Pro-IL-1β, the IL-1β precursor, as a key step in the inflammatory response (3, 13,14). Studies also suggest non-caspase-1 mechanisms can process Pro-IL-1β extracellularly using enzymes like proteinase-3, during neutrophilic inflammation (15). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (16-18), but evidence suggests that these factors can be secreted by non-classical pathways (19, 20). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (21). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (20, 22). 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 the receptor agonist IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) and 68 kDa transmembrane type II receptor (IL-1 RII) share approximately 28% homology in their extracellular domains but play significantly different roles in the inflammatory response. IL-1 RI has a 213 aa cytoplasmic domain that initiates the inflammatory response after IL-1β and IL-1β binding. IL-1RII has a 29 aa cytoplasmic domain and functions as a decoy receptor that suppresses IL-1 signaling by sequestering IL-1α and IL-1β (23, 24). The IL-1 Receptor Accessory Protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (25). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (26, 27).

The Quantikine® HS Mouse IL-1β/IL-1F2 Immunoassay is a 4.0 hour solid phase ELISA designed to measure mouse IL-1β levels in serum and plasma. It contains *E. coli*-expressed recombinant mouse IL-1β and antibodies raised against the recombinant protein. Results obtained using natural IL-1β showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that the Quantikine® HS Immunoassay 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 and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for mouse IL-1β is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-1β HS Microplate	899461	96 well polystyrene microplate (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 zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IL-1β HS Standard	899463	2 vials of recombinant mouse IL-1β in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after use.
Mouse IL-1β HS Conjugate	899462	21 mL of a polyclonal antibody specific for mouse IL-1β conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Streptavidin Polymer-HRP Diluent	898387	21 mL of a solution with preservatives.	
Streptavidin Polymer-HRP (100X)	898350	0.3 mL of Streptavidin Polymer-HRP in a buffer with preservative.	
Plate Sealers	N/A	Adhesive strips.	

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

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Mouse IL-1β Controls (optional; R&D Systems®, Catalog # QC254).

PRECAUTIONS

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS 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.

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 is not validated for use in this assay.*

SAMPLE PREPARATION

Mouse samples may need dilution due to high endogenous values. Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

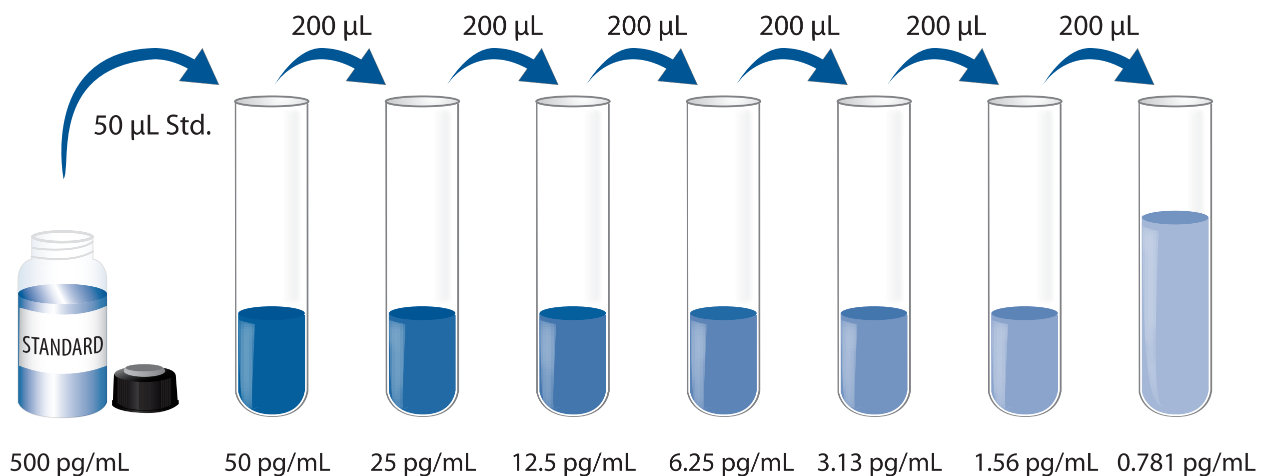
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Streptavidin Polymer-HRP (1X) - Add 0.215 mL of Streptavidin Polymer-HRP (100X) directly to the Streptavidin Polymer-HRP Diluent. Mix well.

Mouse IL-1β HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-1β HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Pipette 450 μL of Calibrator Diluent RD5-3 into the 50 pg/mL tube. Pipette 200 μL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 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 standards and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in 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 RD1-38 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. 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 (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 200 µL of Mouse IL-1β HS Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 200 µL of Streptavidin Polymer-HRP (1X) to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for **30 minutes** at room temperature **on the benchtop. Protect from light.**
11. Add 50 µL 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. 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

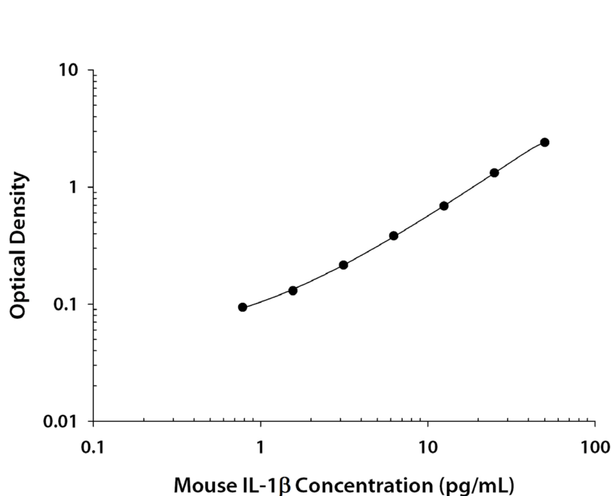
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 measured concentrations 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	0.039 0.044	0.042	—
0.781	0.090 0.097	0.094	0.052
1.56	0.128 0.132	0.130	0.088
3.13	0.214 0.217	0.216	0.174
6.25	0.383 0.384	0.384	0.342
12.5	0.689 0.690	0.690	0.648
25	1.321 1.322	1.322	1.280
50	2.366 2.445	2.406	2.364

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	4.64	14.1	28.5	4.89	14.5	28.9
Standard deviation	0.130	0.378	0.590	0.413	0.796	1.60
CV (%)	2.8	2.7	2.1	8.4	5.5	5.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	95	87-101%
EDTA plasma (n=4)	98	91-105%
Heparin plasma (n=4)	92	84-99%

LINEARITY

To assess the linearity of the assay, samples 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.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	104	103	103
	Range (%)	102-107	97-108	98-106
1:4	Average % of Expected	107	104	107
	Range (%)	101-112	96-109	103-111
1:8	Average % of Expected	112	102	108
	Range (%)	104-118	96-106	103-112
1:16	Average % of Expected	111	96	100
	Range (%)	107-116	88-102	95-104

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SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of mouse IL-1β ranged from 0.058-0.312 pg/mL. The mean MDD was 0.142 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 (aa 118-269) produced at R&D Systems®.

The Non-WHO mouse IL-1β Reference Material for 93/668 (murine rDNA derived) was evaluated in this kit.

The dose response curve of this reference material parallels the Mouse IL-1β HS Standard curve. To convert sample values obtained with the Quantikine® HS Mouse IL-1β kit to approximate NIBSC (93/668) units, use the equation below.

Non-WHO NIBSC (93/668) approximate value (U/mL) = 0.5908 x Quantikine® Mouse HS IL-1β value (pg/mL)

Note: *Based on data generated in April 2019.*

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-1β in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
EDTA plasma (n=10)	2.93	80	ND-6.63

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	10.4	2.65-61.8	18.2
Heparin plasma (n=10)	6.36	1.19-20.5	6.99

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 at 50 ng/mL in a mid-range recombinant mouse IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-1α
IL-1R1
IL-1RII
IL-1ra
IL-1 Rrp2
IL-1RAPL2
IL-18
IL-18 Rα
IL-18 Rβ
IL-33
IL-36 Rα
IL-36α
IL-36β
IL-36γ
IL-38
SIGIRR
ST2

Other recombinants:

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

Recombinant rat IL-1β does not interfere but does cross-react approximately 6.76% in this assay.

Recombinant cotton rat IL-1β interferes at concentrations > 2.0 ng/mL and cross-reacts approximately 0.12% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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NOTES

NOTES

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