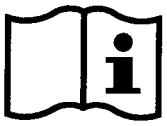
Product information



Userś Manual

Mouse IL-1α ELISA

Sandwich Enzyme-Linked Immunosorbent Assay for the detection of Mouse IL-1α

REF **IB99536**

Σ **96**

Storage: 2-8°C

RUO **For research use only, not for use in diagnostic procedures.**

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1. Introduction

Interleukin-1 alpha (IL-1α) is a protein of the interleukin-1 family that in humans is encoded by the *IL1A* gene. In general, Interleukin 1 is responsible for the production of inflammation, as well as the promotion of fever and sepsis. IL-1α inhibitors are being developed to interrupt those processes and treat diseases. IL-1α is produced mainly by activated macrophages, as well as neutrophils, epithelial cells, and endothelial cells. It possesses metabolic, physiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. It binds to the interleukin-1 receptor. It is on the pathway that activates tumor necrosis factor-alpha. IL-1α is a cytokine of the interleukin-1 family. IL-1α is a unique member in the cytokine family in the sense that the structure of its initially synthesized precursor does not contain a signal peptide fragment (same is known for IL-1β and IL-18). After processing by the removal of N-terminal amino acids by specific proteases, the resulting peptide is called "mature" form. Calpain, a calcium-activated cysteine protease, associated with the plasma membrane, is primarily responsible for the cleavage of the IL-1α precursor into a mature molecule. Both the 31kDa precursor form of IL-1α and its 18kDa mature form are biologically active. The 31 kDa IL-1α precursor is synthesized in association with cytoskeletal structures (micro-tubules), unlike most proteins, which are translated in the endoplasmic reticulum. The three-dimensional structure of the IL-1α contains an open-ended barrel composed entirely of beta-pleated strands. Crystal structure analysis of the mature form of IL-1α shows that it has two sites of binding to IL-1 receptor. There is a primary binding site located at the open top of its barrel, which is similar but not identical to that of IL-1β.

2. Principles of Method

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to mouse IL-1α. Samples are pipetted into these wells. Nonbound mouse IL-1α and other components of the sample should be removed by washing, then polyclonal antibody specific to mouse IL-1α added. In order to quantitatively determine the amount of mouse IL-1α present in the sample, streptavidin Horseradish Peroxidase (HRP) should be added to each microplate well. The final step, a TMB-substrate solution added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450nm. Since the increases in absorbency is directly proportional to the amount of captured mouse IL-1α.

3. Intended Use

The IBL-AMERICA mouse IL-1α ELISA kit is to be used for the determination of mouse IL-1α in mouse serum, mouse plasma, cell lysate, culture supernatants and buffered solution. The assay will recognize native and recombinant mouse IL-1α.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -70°C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- Standard protein and 2nd antibody containing Sodium Azide as a preservative.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Washing Buffer	2	(20X) 25 ml
Standard Protein	1 Glass vial (lyophilized)	
Standard/Sample Dilution Buffer	1	25 ml
Secondary Antibody	1 Glass vial (lyophilized)	
Streptavidin HRP(X100)	1	150 µl
Secondary antibody/ streptavidin HRP Dilution Buffer	1	25 ml
Substrate (TMB)	1	15 ml
Stop Solution	1	15 ml
Protocol booklet	1	
Plate sealers	2	

① 96 Well Plate
: Mouse IL-1α microtiter plate, one plate of 96 wells (8 well strips x 12).
A plate using break-apart strips coated with a mouse monoclonal antibody specific to mouse IL-1α.

② Standard Protein
: Recombinant mouse IL-1α.

③ Secondary Antibody
: Biotinylated anti mouse IL-1α antibody.

④ Streptavidin HRP(X100)
: Streptavidin Horseradish Peroxidase (HRP, enzyme)

⑤ Substrate (Stabilized chromogen)
: Tetramethylbenzidine (TMB) solution

⑥ Stop Solution
: 1N solution of sulfuric acid (H_2SO_4).

⑦ Plate sealer
: Adhesive sheet.

- Do not mix or interchange different reagents from various kit lots.

7. Materials Required But Not Provided

① Microtiter plate reader capable of measurement at or near 450nm.
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 Data analysis and graphing software
⑤ Vortex mixer
⑥ Polypropylene tubes for diluting and aliquoting standard
⑦ Absorbent paper towels
⑧ Calibrated beakers and graduated cylinders of various sizes

8. Reagent Preparation

1) Mouse IL-1α standard

1. Reconstitute the lyophilized mouse IL-1α standard by adding 1 ml of *Standard/Sample Dilution Buffer* to make the 1 ng/ml standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting.
2. Prepare 1 ml of 100 pg/ml top standard by adding 100 µl of the above stock solution in 900 µl of *Standard/Sample Dilution Buffer*. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (1.56 pg/ml ~ 100 pg/ml) as below. *Standard/Sample Dilution Buffer* serves as the zero standard (0 pg/ml).

Standard	Add	Into
100 pg/ml	*100 µl of the std.(1ng/ml)*	900.0 µl of the *Standard/Sample Dilution Buffer*
50 pg/ml	*500 µl of the std.(100pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
25 pg/ml	*500 µl of the std.(50pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
12.5 pg/ml	*500 µl of the std.(25pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
6.25 pg/ml	*500 µl of the std.(12.5pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
3.125 pg/ml	*500 µl of the std.(6.25pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
1.56 pg/ml	*500 µl of the std.(3.125pg/ml)*	500.0 µl of the *Standard/Sample Dilution Buffer*
0 pg/ml	1.0 ml of the *Standard/Sample Dilution Buffer*	

2) Secondary Antibody

100X secondary antibody solution can be made by adding 150 µl *Secondary antibody/ Streptavidin HRP dilution buffer* in the vial.

1. Equilibrate to room temperature, mix gently.

2. Mix 20 µl *Secondary Antibody concentrated solution* (100X) + 1.98 ml *Secondary antibody/ Streptavidin HRP dilution buffer*. (Sufficient for one 16-well strip, prepare more if necessary)
   Label as "Working Secondary antibody Solution".
3. Return the unused *Secondary Antibody concentrated solution* to the refrigerator.

3) Streptavidin HRP(X100)

1. Equilibrate to room temperature, mix gently.
2. Mix 20 µl *Streptavidin HRP concentrated solution* (100X) + 1.98 ml *Secondary antibody/ Streptavidin HRP dilution buffer*. (Sufficient for one 16-well strip, prepare more if needed)
   Label as "Working Streptavidin HRP Solution".
3. Return the unused *Streptavidin HRP concentrated solution* to the refrigerator.

4) Washing buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 0.5 volume *Wash buffer concentrate solution* (20X) + 9.5 volumes of deionized water. Label as "Working Washing Solution".
3. Store both the concentrated and the Working Washing Solution in the refrigerator.

*** Directions for washing**

1. Fill the wells with 300 µl of "Working Washing Buffer".
   Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.
   If using an automated washer, the operating instructions for washing equipment should be carefully followed.
2. Incomplete washing will adversary affects the assay and renders false results.
3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

5) Sample preparation

Blood should be collected by venipuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Samples may be shipped at room temperature and then stored refrigerated at 2-8℃ if testing is to take place within one week after collection. If testing is to take place later than one week, samples should be stored at -20℃. Avoid repeated freeze/thawing.

9. Assay Procedure

- Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
- All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- A standard curve must be run with each assay.
- If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.

1) Determine the number of 16-well strips needed for assay. Insert these in the flame(s) for current use (Re-bag extra strips and frame. Refrigerate for further use).
2) For the standard curve, add 100 µl of the standard to the appropriate microtiter wells. Add 100 µl of the *Standard/Sample/secondary antibody Dilution Buffer* to zero wells.
3) Serum and plasma require appropriate fold dilution in the *Standard/Sample Dilution Buffer.* And add 100 µl of samples to each wells.
4) Cover the plate with the plate cover and incubate for 2 hours at 37℃.
5) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
6) Pipette 100 µl of "Working Secondary Antibody Solution" into each well.
7) Cover the plate with the plate cover and incubate for 1 hour at 37℃.
8) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
9) Add 100 µl "Working Streptavidin HRP Solution" to each well.
10) Cover the plate with the plate cover and incubate for 30 minutes at 37℃.
11) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
12) Add 100 µl of *Substrate* to each well. The liquid in the wells should begin to turn blue.
13) Incubate the plate at room temperature.

- Do not cover the plate with aluminum foil, or color may develop.
  The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450nm can only be read after the Stop Solution has been added to each well.
- Because the *Substrate* is light sensitive, avoid the remained *Substrate* solution prolonged exposure to light.

- Typically, reaction is stopped 10~15 minutes after treatment of Substrate, but this time can be adjusted as the user desires.

14) Add 100 µl of *Stop Solution* to each well. The solution in the wells should change from blue to yellow.

15) Read the absorbance of each well at 450nm. Read the plate within 20 minutes of adding the *Stop Solution*.

16) Plot on graph paper the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.

17) Read the mouse IL-1α concentrations for the unknown samples and controls from the standard curve plotted in step 18. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest standard should be further diluted in the S*tandard/Sample Dilution Buffer*).

10. Characteristics

1) Typical result

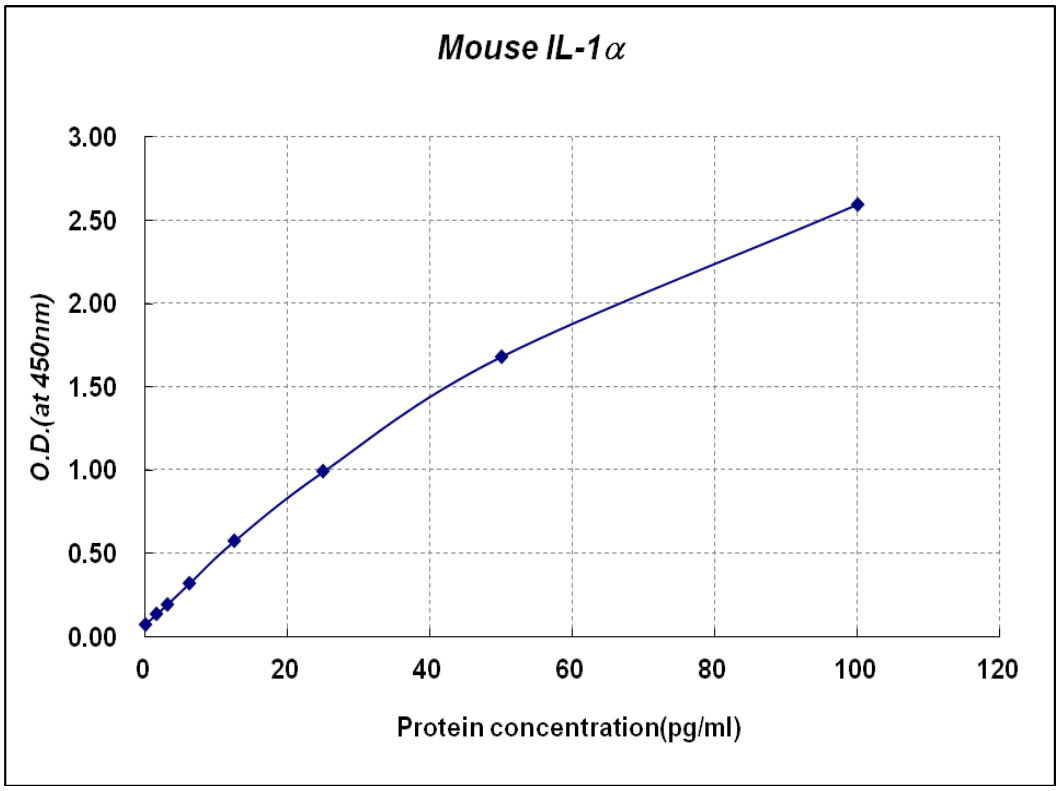
The standard curve below is for illustration only and **should not be used** to calculate results in your assay.

A standard curve must be run with each assay.

Standard Mouse IL-1α(pg/ml)	Optical Density (at 450nm)
0	0.075
1.5625	0.135
3.125	0.196
6.25	0.323
12.5	0.575
25	0.992
50	1.683
100	2.598

< Limitations >

- Do not extrapolate the standard curve beyond the 100 pg/ml standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native mouse IL-1α in various matrices has not been investigated.



(TMB reaction incubate at room temperature for 10 min)

2) Sensitivity

The minimal detectable dose of mouse IL-1α was calculated to be 0.356 pg/ml, by subtracting three standard deviations from the mean of 12 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation..

N	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
ZERO	**0.0792**	**0.0742**	**0.0716**	**0.0761**	**0.0745**	**0.0746**	**0.0792**	**0.0742**	**0.0716**	**0.0761**	**0.0745**	**0.0746**

Average	**SD**	**LLD**	**LLD mean(pg/ml)**
0.0750	**0.00239**	**0.0822**	**0.356**

3) Specificity

The following substances were tested and found to have no cross-reactivity: human IL-1α, rat IL-2, mouse IL-1β, human IL-1β, mouse IL-6, mouse IL-8, mouse IL-15, mouse IFN−γ, mouse TNF−α

4) Precision

① Within-Run (Intra-Assay)

(n=12)

Mean (pg/ml)	SD	CV (%)
12.720	0.492	3.868
24.866	0.813	3.271
50.088	0.975	1.947
100.198	2.635	2.630

② Between-Run (Inter-Assay)

(n=12)

Mean (pg/ml)	SD	CV (%)
12.063	0.251	2.084
24.340	0.387	1.588
48.765	0.750	1.538
100.102	0.749	0.748

5) Recovery

Recovery on addition is 87.884~98.183% (mean 92.866%)

Added Analyte (pg/ml)	Serum(1/4)+added analyte (450nm)	Serum 1/4(450nm) +added analyte(450nm)	Recovery (%)
12.594	16.058	14.113	87.884
24.573	28.615	26.078	91.132
50.369	56.039	55.020	98.183
99.858	109.826	103.527	94.265

11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much streptavidin-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30℃ before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing –unbound streptavidin-HRP remaining	• Increase number of washes carefully
	• Too much streptavidin-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual streptavidin -HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12. Reference

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◈ Ordering Information

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