Product information

User's Manual

IL-1α mouse ELISA

Enzyme immunoassay (ELISA) for the determination of mouse IL-1α in cell culture supernatants, serum and plasma.

REF IB99509

Σ 96

Storage: 2-8°C

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

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1. Intended Use

The IL-1 α mouse ELISA Kit is to be used for the measurement of mouse IL-1α in cell culture supernatants, serum and plasma. This ELISA kit is for research use only.

2. Introduction

The IL-1 family consists of two pro-inflammatory cytokines IL-1α, IL-1β and the anti-inflammatory IL-1 receptor antagonist (IL-1Ra or IL-1RN). The two IL-1 isoforms (α and β) are encoded by distinct genes but are structurally related and bind to the same receptors that exists in two major forms, Type I (IL-1R1) and Type II (IL-1R2) (1). The major biological roles of IL-1 are described as endogenous pyrogen, leukocyte endogenous mediator, lymphocyte, β-cell, osteoclast, epidermal cell derived thymocyte activating factor, hematopoietin-1 and mononuclear cell factor (1). IL-1α plays a central role in cell growth, tissue repair and chronic inflammatory diseases (1).

IL-1α is a unique member in the cytokine family which is synthesized as a 33 kDa precursor protein, ProIL-1α (1). The mature form p17 results by removal of N-terminal amino acids from ProIL-1α. Recently, it has been shown that the mature form p17 of IL-1α is the active cytokine. The ProIL-1α is poorly active due to its binding to the inhibitory intracellular IL-1R2 protein. Upon inflammasome activation, caspase-1 specifically cleaves IL-1R2, causing release of ProIL-1α followed by its processing into a 17 kDa mature form by the calcium-dependent, membrane-associated cysteine protease, called calpain (3). Mature IL-1α is released into the extracellular compartment while the IL-1α N-terminal prodomain (1–115 amino acids) is located in the nucleus (1). The proinflammatory properties of IL-1α may be relevant to the development of atherosclerotic vascular disease. IL-1α also has powerful effects on adaptive immunity by enhancing expansion and survival of T cells, differentiation of T helper 17 (Th17) cells and effector T cell proliferation in the presence of regulatory T cells (2).

IL-1α is not commonly found in the circulation or in body fluids, except if the cytokine might be released from dying cells (4) or secreted upon inflammasome activation (5). Increased IL-1α activity is a hallmark of many chronic inflammatory conditions, including rheumatoid arthritis, gout, diabetes, atherosclerosis and psoriasis (1).

3. General References

(1) Immunological and inflammatory functions of the interleukin-1 family: C.A. Dinarello; Annu. Rev. Immunol. **27,** 519 (2009)

(2) The IL-1 family: regulators of immunity: J.E. Sims & D.E. Smith; Nat. Rev. Immunol. **10,** 89 (2010)

(3) Intracellular interleukin-1 receptor 2 binding prevents cleavage and activity of interleukin-1a, controlling necrosis-induced sterile inflammation: Y. Zheng, et al.; Immunity **38,** 285 (2013)

(4) Identification of a key pathway required for the sterile inflammatory response triggered by dying cells: C.J. Chen, et al.; Nat. Med. **13,** 851 (2007)

(5) Inflammasome activators induce interleukin-1α secretion via distinct pathways with differential requirement for the protease function of caspase-1: O. Gross, et al.; Immunity **36,** 388 (2012)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for the measurement of mouse IL-1alpha (IL-1α) in cell culture supernatants, serum and plasma. A monoclonal antibody specific for IL-1α has been pre-coated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, IL-1α is recognized by the addition of a biotinylated monoclonal antibody specific for IL-1α (DET). After removal of excess biotinylated antibody, streptavidin-peroxidase (STREP-HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of IL-1α in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

• 1 vial mouse IL-1α Standard (lyophilized)	(1 µg)	(STD)
• 1 vial Detection Antibody	(20 µl)	(DET)
• 1 vial HRP Labeled Streptavidin (lyophilized)	(2 µg)	(STREP-HRP)
• 2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
• 2 bottles ELISA Buffer 10X	(2 x 30 ml)	(ELISA Buffer 10X)
• 1 bottle TMB Substrate Solution	(12 ml)	(TMB)
• 1 bottle Stop Solution	(12 ml)	(STOP)
• 1 plate coated with mIL-1α Antibody	(6 x 16-well strips)	
• 2 plate Covers (plastic film)		
• 2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips.
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: *Prepare just the appropriate amount of the buffers necessary for the assay.*

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.
- **ELISA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.
- **Detection Antibody (DET)** has to be diluted to 1:2000 in ELISA Buffer 1X (2 µl DET + 4 ml ELISA Buffer 1X).

  NOTE: *The diluted Detection Antibody is not stable and cannot be stored!*

- **HRP Labeled Streptavidin (STREP-HRP)** has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**.
  - Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).

  NOTE: *The diluted STREP-HRP is not stable and cannot be stored!*

- **Mouse IL-1α Standard (STD)** has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - This reconstitution produces a stock solution of 10 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

  NOTE: *The reconstituted standard is aliquoted and stored at -20°C!*

  - Dilute the standard protein concentrate (STD) (**10 µg/ml**) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended.
  - Suggested standard points are:

    1000, 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/ml.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
100 ng/ml	10 µl of IL-1α (STD) (10 µg/ml)	990 µl of ELISA Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
1000 pg/ml	10 µl of IL-1α (100 ng/ml)	990 µl of ELISA Buffer 1X
500 pg/ml	300 µl of IL-1α (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of IL-1α (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml	300 µl of IL-1α (250 pg/ml)	300 µl of ELISA Buffer 1X
62.5 pg/ml	300 µl of IL-1α (125 pg/ml)	300 µl of ELISA Buffer 1X
31.2 pg/ml	300 µl of IL-1α (62.5 pg/ml)	300 µl of ELISA Buffer 1X
15.6 pg/ml	300 µl of IL-1α (31.2 pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

8.2. Sample collection, storage and dilution

Cell Culture Supernatants, serum and plasma have to be diluted in ELISA Buffer 1X. Starting dilutions of 1/2 to 1/20 are recommended.

8.3. Assay Procedure (Checklist)

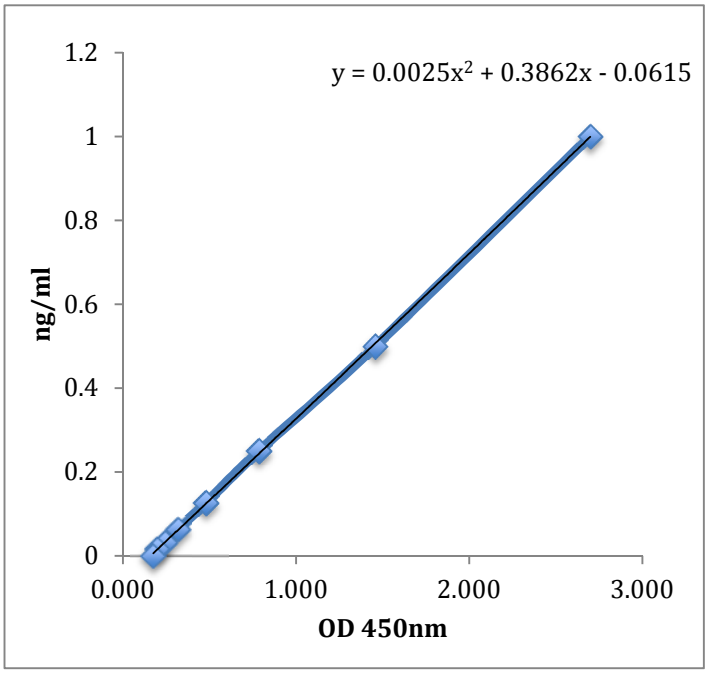
☐	1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and stored at 4°C. **NOTE:** *Remaining 16-well strips coated with* IL-1α *antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells **(see 8.1. Preparation and Storage of Reagents and 8.2 Preparation of Samples).**
☐	3. Cover the plate with plastic film and incubate for **2 h at room temperature** (RT).
☐	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	5. Add 100 µl to each well of the diluted Detection Antibody **(DET) (see 8.1 Preparation and Storage of Reagents)**.
☐	6. Cover the plate with plastic film and incubate for **1 h at RT**.
☐	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	8. Add 100 µl to each well of the diluted HRP Labeled Streptavidin **(STREP-HRP) (see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plastic film and incubate for **30 min at RT**.
☐	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	11. Add 100 µl to each well of TMB substrate solution **(TMB)**.
☐	12. Allow the color reaction to develop **at RT in the dark for 10-20 minutes**. Do not cover the plate.
☐	13. Stop the reaction by adding 50 µl of Stop Solution (**STOP**). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.
	! CAUTION: CORROSIVE SOLUTION !
☐	14. Measure the OD at 450 nm in an ELISA reader.

9. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding IL-1α concentration (pg/ml) on the vertical axis (see **10.** TYPICAL DATA).
- Calculate the IL-1α concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of mouse IL-1α in the sample.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:



Standard IL-1α (ng/ml)	Optical Density (mean)
1	2.698
0.5	1.457
0.25	0.785
0.125	0.476
0.0625	0.314
0.03125	0.242
0.0156	0.197
0	0.175

Figure: Standard curve

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of IL-1α that can be detected by this assay is 9 pg/ml. **NOTE:** *The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.*

B. Assay range: 15.6 pg/ml – 1000 pg/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant mouse IL-1α (proform and p17 fragment). It does not cross-react with human IL-1α or mouse IL-β.

D. Intra-assay precision:

Four samples of known concentrations of mouse IL-1α were assayed in replicates 7 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV (%)	n
A1	9.49	0.129	1.35	7
A2	0.30	0.003	0.87	7
A3	1.20	0.016	1.30	7
A4	2.40	0.015	1.30	7

E. Inter-assay precision:

Four samples of known concentrations of mouse IL-1α were assayed in 3 separate assays to test precision between assays.

Samples	Means (ng/ml)	SD	CV (%)	n
B1	2.5	0.10	4.10	3
B2	7.95	0.209	2.71	3
B3	1.38	0.016	1.20	3
B4	0.412	0.024	5.81	3

F. Linearity:

Different samples containing mouse IL-1α were diluted several fold (1/2 to 1/20) and the measured recoveries ranged from 95% to 105%.

G. Expected values:

Mouse IL-1α levels range in cell culture supernatant of bone marrow-derived dendritic cells (BMDCs) activated by Nigericin from **5 to >10ng /ml**.

12. Technical Hints and Limitations

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Solution protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes

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