Mouse IL-1α ELISA Kit

For the quantitative determination of mouse interleukin-1α (IL-1α) concentrations in mouse serum, cell culture supernatant, and other biological fluids

MEC1011-2 *(192 tests × 1)*
MEC1011-6 *(192 tests × 3)*

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (01/15-02) mIL-1α [2/6]

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INTENDED USE

This Mouse IL-1α ELISA kit is to be used for the *in vitro* quantitative determination of mouse interleukin 1α (IL-1α) concentrations in serum, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

INTRODUCTION

IL-1α is a member of interleukin 1 family. IL-1α and IL-1β recognize the same IL-1 receptor and share a number of similar biological functions. IL-1α is predominantly a cell-associated molecule whereas IL-1β is a secreted molecule. IL-1α is synthesized primarily as a precursor that is biologically active via specific cell binding. Precursor IL-1α can be cleaved by extracellular proteases when the cells die, and can also be cleaved by activation of the calcium-dependent, membrane-associated calpains. Cleavage of the mouse IL-1α precursor results in an 18 kDa mature mouse IL-1α molecule.

IL-1α is constitutively expressed by epithelial cells and the essential role of IL-1α in maintenance of skin barrier function. IL-1α can also produced by macrophages and neutrophils by the induction of microbes and microbial products. IL-1α can induce its own synthesis as well as the production of TNF and IL-6. IL-1α induces the production of IL-2, IL-2 receptors, GM-CSF and IL-4 from activated T cells, stimulates B cell proliferation and maturation, and increases immunoglobulin synthesis. IL-1α affects NK cell activation and LAK production associated with other cytokines, and induces prostaglandin synthesis in endothelial cells and smooth muscle cells, collagenase production in synovial cells, and cartilage and calcium resorption in bones.

Studies have shown a connection between IL-1α and the pathogenesis of endometriotic lesions. The increased expression of both matrix-degrading MMP-1 and its major stimulatory cytokine IL-1α in endometriotic lesions and the selective co-expression in the stroma of endometriotic foci clearly suggests the involvement of the IL-1α molecule in the pathogenic mechanisms leading to local invasion and tissue destruction. Reports also indicate that the translation of the neurotransmitter gene only occurs after receiving IL-1α stimulation. This effect was suppressed by co-stimulation with IL-1 receptor antagonist. High levels of IL-1α are associated with sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, and atherosclerosis.

PRINCIPLE OF THE ASSAY

This mouse IL-1α enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for mouse IL-1α. When standards or samples are added to the appropriate microtiter plate wells, mouse IL-1α in the

standards or samples will be immobilized by the precoated antibody during incubation. Then, a biotin-conjugated antibody preparation specific for mouse IL-1α is added to each well and incubated. The biotin labelled antibody attaches to the wells by binding to mouse IL-1α. After plate washing, other proteins, components and unattached biotin labelled antibody is removed. After that, avidin-horseradish peroxidase (HRP) conjugate is added to each well. Avidin has a very high affinity for biotin, thus, it links the tracer (HRP) sturdily to the biotin labelled antibody. The wells are thoroughly washed to remove all unbound avidin-HRP conjugate and a TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only wells that contain mouse IL-1α will exhibit a change in colour. The extent of colour change is proportional to the quantity of mouse IL-1α present in the standards/samples. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wave length of 450 nm ± 2 nm.

In order to measure the concentration of mouse IL-1α in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus IL-1α concentration (pg/mL). The concentration of mouse IL-1α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This mouse IL-1α ELISA is a 3.5-hour solid-phase immunoassay readily applicable to measure mouse IL-1α levels in serum, cell culture supernatant, and other biological fluids in the range of 0 to 500pg/mL.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	192 tests × 1	***192 tests × 3***

1. **MOUSE IL-1α MICROTITER PLATE** (Part MEC11-1) ***96 wells × 2*** ***96 wells × 6***
   Pre-coated with anti-mouse IL-1α monoclonal antibody

2. **BIOTIN CONJUGATE** (Part MEC11-2) ***12 mL*** ***12 mL × 3***
   Anti-mouse IL-1α monoclonal antibody conjugated to biotin

3. **AVIDIN-HRP CONJUGATE** (Part MEC11-3) ***24 mL*** ***24 mL × 3***
   Avidin conjugated to horseradish peroxidase

4. **MOUSE IL-1α STANDARD** (Part MEC11-4) ***4 vials*** ***4 vials × 3***
   Recombinant mouse IL-1α (500pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC11-5) ***50 mL*** ***50 mL × 3***
   Animal serum with preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part MEC11-6) ***50 mL*** ***50 mL × 3***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (30X)** (Part 30009) ***60 mL*** ***60 mL × 3***
   30-fold concentrated solution of buffered surfactant.

8. **TMB SUBSTRATE:** (Part 30010) ***20 mL*** ***20 mL × 3***
   Ready to use

9. **STOP SOLUTION** (Part 30008) ***28 mL*** ***28 mL × 3***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL required for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate mouse viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Crystal formation in the wash buffer (30X) will not affect its quality. Prior to dilution, make sure all crystals are completely dissolved by warming to 37°C.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid hemolytic, lipidic or turbid samples.
- Samples must be stored at -20°C (short term) or -70°C (long term) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- Serum and cell culture supernatant are to be thawed immediately before use.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

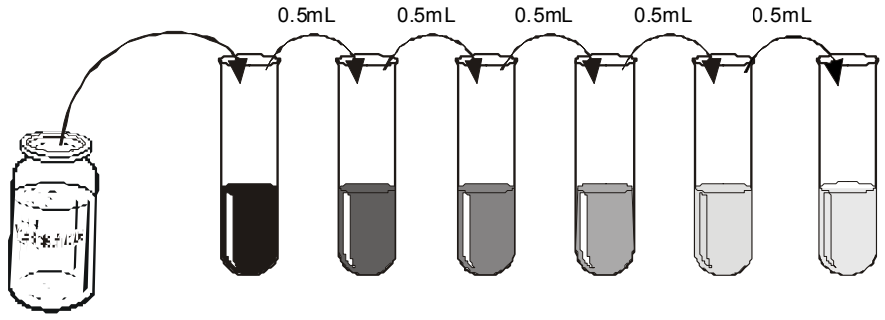
1. **Wash Buffer (1X):** Dissolving the crystals in Wash Buffer (30X) by warming up to 37°C. Add 60 mL of Wash Buffer (30X) and dilute to a final volume of 1800 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (30X) to 29 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Mouse IL-1α Standard:**

   a) Two vials of Standards are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute the mouse IL-1α Standard with either 1.0mL of Calibrator Diluent I (for serum testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 500pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. Aliquot the stock and store frozen at -20°C (short term) or -70°C (long term) if repeated use is expected.

   b) Use the above stock solution to produce a 2-fold serial dilution within the range of this assay (7.8pg/mL to 500pg/mL) as illustrated. Add 0.5mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-1α standard will serve as the high

standard (500pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).



500pg/mL 250pg/mL 125pg/mL 62.5pg/mL 31.25pg/mL 15.6pg/mL 7.8pg/mL

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse IL-1α Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0pg/mL** (S1)	**2A, 2B**	Standard 5 – **62.5pg/mL** (S5)
1C, 1D	Standard 2 – **7.8pg/mL** (S2)	**2C, 2D**	Standard 6 – **125pg/mL** (S6)
1E, 1F	Standard 3 – **15.6pg/mL** (S3)	**2E, 2F**	Standard 7 – **250pg/mL** (S7)
1G, 1H	Standard 4 – **31.25pg/mL** (S4)	**2G,2H**	Standard 8 – **500pg/mL** (S8)
		3A-12H	mouse IL-1α **samples**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50μL of Standard or sample to the appropriate well of anti-mouse IL-1α antibody pre-coated microtiter plate. Cover and incubate for 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL anti-mouse IL-1α biotin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μL of Avidin-HRP conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Repeat wash procedure as described in Step 4.

7. Add 100μL TMB to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of mouse IL-1α in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-1α concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-1α in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-1α concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

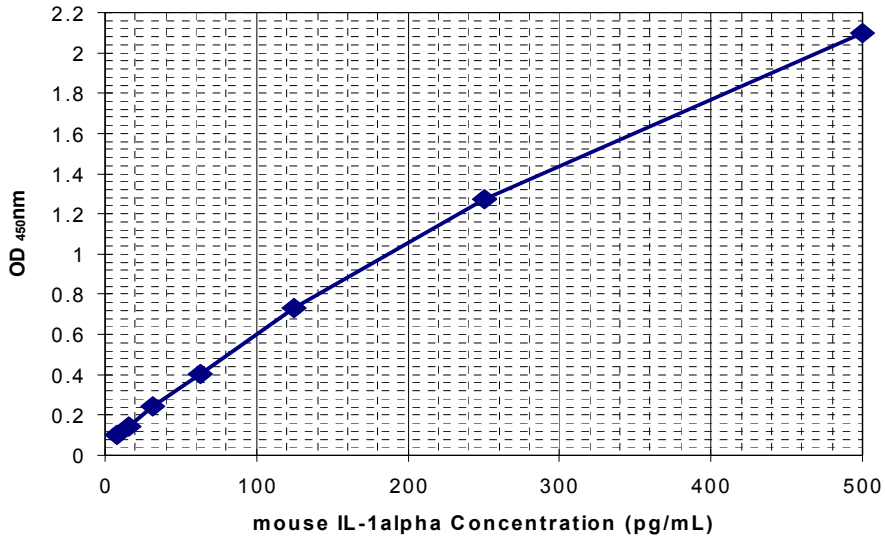
TYPICAL DATA

Results of a typical standard run of a mouse IL-1α ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

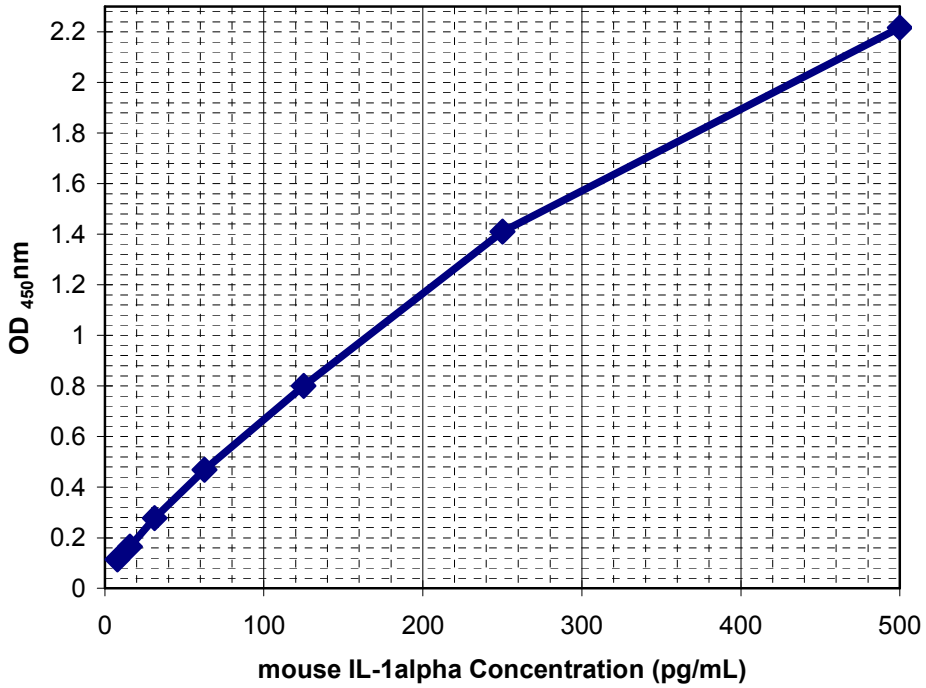
Standard (pg/mL)	**Mean O.D. (450 nm)**	**%CV**	**Zero Standard Subtracted** (Std.)-(S1)
0	0.0570	9.92	0
7.8	0.0995	0.71	0.0425
15.6	0.1435	3.45	0.0865
31.25	0.2415	1.46	0.1845
62.5	0.4025	1.76	0.3455
125	0.7325	2.03	0.6750
250	1.2710	0.11	1.2140
500	2.0995	0.30	2.0425



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.)-(S1)
0	0.0600	7.07	0
7.8	0.1145	6.79	0.0545
15.6	0.1655	6.41	0.1055
31.25	0.2765	4.35	0.2165
62.5	0.4695	2.86	0.4095
125	0.8000	6.01	0.7400
250	1.4095	3.86	1.3495
500	2.2160	2.04	2.1560



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

Sample	1	2	3
N	16	16	16
Mean (pg/ml)	234.32	119.37	33.55
Coefficient of Variation (%)	5.81	7.60	6.33

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were measured by using replicates on 8 different assays with **calibrator II as standard diluent.**

Sample	1	2	3
N	8	8	8
Mean (pg/mL)	132.32	62.78	16.20
Coefficient of Variation (%)	5.53	3.80	7.19

3. RECOVERY

The recovery of mouse 1α within cell culture media and mouse serum was evaluated with mouse 1α spiked samples.

Sample Type	Average Recovery %	Range %
Cell culture media	92.7	92 -93
Mouse Serum	77	66-87

4. SENSITIVITY

The minimum detectable dose of mouse IL-1α was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse IL-1α calculated from calibrate II diluted standard curve was <0.8pg/mL.

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