

Human IL-1 α ELISA Kit

For the quantitative determination of human Interleukin-1 alpha (IL-1 α) concentrations in serum, plasma, and cell culture supernatant.

Catalogue Number: EL10040

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE

This Human IL-1 α ELISA kit is to be used for the *in vitro* quantitative determination of human Interleukin1-alpha (IL-1 α) concentrations in serum, plasma, and cell culture supernatant. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin 1 (IL-1) is a name that designates two proteins, IL-1 α and IL-1 β , which are the products of distinct genes, but which recognize the same cell surface receptors. With the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system, IL-1 is not produced by the cells of healthy individuals. However, in response to stimuli such as those produced by inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is observed.

IL-1 α and IL-1 β are structurally related polypeptides that show approximately 25% homology at the amino acid level (31). Both are synthesized as 31 kDa precursors that are subsequently cleaved into proteins with molecular weights of approximately 17.5 kDa (15,18). Neither IL-1 α nor IL-1 β contains a typical hydrophobic signal peptide sequence (3,27,29), but evidence suggests that these factors can be secreted by non-classical pathways (33,34). A large proportion of IL-1 α is retained intracellularly in its precursor form (12). A portion of this unprocessed IL-1 α is transported to the cell surface and remains associated with the cell membrane (10,12,23). The membrane-bound, unprocessed IL-1 α is apparently biologically active, acting in a paracrine fashion on adjacent cells having IL-1 receptors (10,12). The precursor form of IL-1 β , unlike the IL-1 α precursor, shows little or no biological activity in comparison to the 17.5 kDa processed form (16,17,23,34). Intracellular IL-1 β consists exclusively of the 31 kDa precursor form (18). Extracellular IL-1 β consists of a mixture of both unprocessed and mature IL-1 β . These results indicate that processing takes place subsequent to secretion and is not tightly coupled to secretion (4,18,33,34). The specific protease apparently responsible for the processing of IL-1 β , designated interleukin1 β -converting enzyme (ICE), has been described (4).

IL-1 α and IL-1 β exert their effects by binding to specific receptors. Two distinct receptor types have been isolated that bind both forms of IL-1. An 80 kDa membrane bound receptor protein, IL-1 receptor type I (IL-1 RI), has been isolated from T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (10,12,37). IL-1 RI has been cloned from mouse and human cells (36) and found to be a member of the Ig super family. A second type of IL-1 receptor, IL-1 receptor type II (IL-1 RII), has been found on B cells, neutrophils, and bone marrow cells (10,12). This receptor has an apparent molecular weight of about 68 kDa and is also a member of the Ig super family. 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 amino acid residues, whereas the type I receptor has a cytoplasmic domain of 213 amino acid residues (10,36). In general, IL-1 α binds better to the type I receptor and IL-1 β binds better to the type II receptor (10). At present, the mechanisms involved in the transduction of the signal initiated by binding of IL-1 are not well characterized (10).

IL-1 possesses a wide variety of biological activities. It has been shown to induce prostaglandin synthesis in endothelial cells and smooth muscle cells (6,8). In the liver, IL-1 initiates the acute phase response resulting in an increase in hepatic protein synthesis and decreased albumin production (32). IL-1 induces collagenase production in synovial cells and cartilage and calcium resorption in bones (5,13). Central nervous system effects of IL-1 include fever induction (endogenous pyrogen activity), induction of slow wave sleep, and release of corticotropin-releasing factor and adrenocorticotropin (9,11,35). IL-1 also effects the endocrine system, acting directly on the adrenal glands to induce steroidogenesis (24). In small doses, IL-1 induces insulin production, but in larger doses is cytotoxic to β cells of the pancreas (28). It has been shown to be a hypoglycemic agent in normal mice and genetically altered, insulin-resistant mice (7). IL-1 also plays an important role in immune functions, having effects on macrophages/monocytes, T lymphocytes, B lymphocytes, NK cells, and LAK cells. It acts on macrophages/monocytes, inducing its own synthesis as well as the production of TNF and IL-6 (25,30). It activates T cells, resulting in IL-2 production and expression of IL-2 receptors (21). IL-1 also induces the production of GM-CSF and IL-4 from activated T cells (19). It induces B cell proliferation and maturation and increased immunoglobulin synthesis (1,26). IL-1, in synergy with other cytokines, plays a role in NK cell activation and LAK production, resulting in tumoricidal activity (2,22).

These reported biological effects of IL-1 range from inducing specific cell type responses to targeting entire systems. Although normal production of IL-1 is obviously critical to mediation of normal host responses to injury and infection, inappropriate or prolonged production of IL-1 has been implicated as playing a role in the production of a variety of pathological conditions. These include sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, and atherosclerosis (10,12).

Current methods for the assay of IL-1 α are based on either the dose-dependent stimulation of ³H-thymidine incorporation into PHA-stimulated thymocytes [LAF assay (14)] or proliferation of the murine T-helper cell line D10.G4.1 (20). These assays require 3-4 days for completion and do not distinguish between IL-1 α and IL-1 β . This IL-1 α Immunoassay is a 3.5 hour solid phase ELISA designed to measure IL-1 α in cell culture supernate, serum, and plasma. It contains recombinant human IL-1 α and antibodies raised against recombinant human IL-1 α and has been shown to accurately quantitate the recombinant factor. Results obtained using natural IL-1 α showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this Immunoassay kit can be used to determine relative mass values for natural IL-1 α .

PRINCIPLE OF THE ASSAY

This IL-1 α 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 to IL-1 α . Standards or samples are then added to the appropriate microtiter plate wells and incubated. After washing to remove unbound IL-1 α and other components of the sample, biotin-conjugated polyclonal antibody specific to IL-1 α is added and incubated. IL-1 α , if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by the biotin conjugate. In order to quantitatively determine the amount of IL-1 α present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin 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 those wells that contain IL-1 α , biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm.

In order to measure the concentration of IL-1 α in the samples, this kit includes 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 standard provided 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-1 α concentration (pg/mL). The concentration of IL-1 α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, not for use in diagnostic procedures.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

		96 tests
1.	IL-1α MICROTITER PLATE (Part 40009) _____ Pre-coated with anti-human IL-1 α monoclonal antibody.	96 wells
2.	BIOTIN CONJUGATE (Part 40010) _____ Anti-human IL-1 α polyclonal antibody conjugated to Biotin.	11 mL
3.	AVIDIN CONJUGATE (Part 40011) _____ Avidin conjugated to horseradish peroxidase.	14 mL
4.	IL-1α STANDARD (Part 40012) _____ Recombinant human IL-1 α (2000 pg/vial) in a buffered protein base with preservative, lyophilized.	2 vials
5.	CALIBRATOR DILUENT I (Part 30003) _____ Animal protein with buffer and preservative. <i>For serum/plasma testing.</i>	22 mL
6.	CALIBRATOR DILUENT II (Part 30004) _____ Cell culture medium with animal protein and preservative. <i>For cell culture supernatant testing.</i>	22 mL
7.	WASH BUFFER (20X) (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	60 mL
8.	SUBSTRATE A (Part 30006) _____ Buffered solution with H ₂ O ₂	10 mL
9.	SUBSTRATE B (Part 30007) _____ Buffered solution with TMB.	10 mL
10.	STOP SOLUTION (Part 30008) _____ 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material!	14 mL

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μ L and 50-200 μ L 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 \pm 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. Human 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 human 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 human 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 the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

- a) **Cell Culture Supernatant:** Collect cell culture supernatant, Centrifuge to remove any visible pellets. Assay can be immediately conducted or samples can be aliquoted and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Special caution: The supernatant may contain a certain level of latent IL-1 α if bovine serum is added as a supplement to the media. To achieve best results, avoid using such media or if it is inevitable create an appropriate approach to determine the base line level of IL-1 α .
- b) **Serum:** Use a serum separator tube (SST) and allow samples to clot for one hour at room temperature. For complete release of IL-1 α , incubate overnight at 2-8 $^{\circ}\text{C}$ before centrifugation. Centrifuge for 10 minutes at 1000 x g (4 $^{\circ}\text{C}$). Remove serum and assay (see activation procedure) immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.
- c) **Plasma:** Collect plasma on ice using EDTA as an anticoagulant. Centrifuge at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 $^{\circ}\text{C}$ is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at $\leq -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

PREPARATION OF REAGENTS

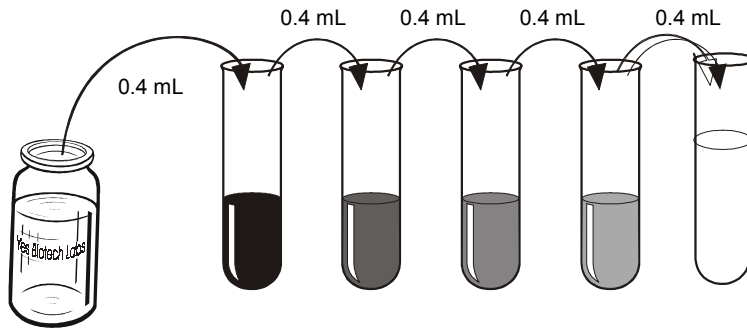
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 $^{\circ}\text{C}$). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8 $^{\circ}\text{C}$. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-1 α Standard:**

- a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-1 α Standard with either 4mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 500 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-1 α standard stock solution must be stored frozen (-20°C) immediately after use so that it can last for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.
- b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (0 pg/mL to 500 pg/mL) as illustrated. Add 0.4 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-1 α Standard will serve as the **high standard (500 pg/mL)** and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-1 α Standard	250	125	62.5	31.3	15.6
500 pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-1 α Standards before starting assay procedure. *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 - 0 pg/mL (S1)	2A, 2B	Standard5 - 125 pg/mL (S5)
1C, 1D	Standard 2 - 15.6 pg/mL (S2)	2C, 2D	Standard6 - 250 pg/mL (S6)
1E, 1F	Standard 3 - 31.3 pg/mL (S3)	2E, 2F	Standard7- 500 pg/mL (S7)
1G, 1H	Standard4 - 62.5 pg/mL (S4)	2G, 12H	IL-1 α samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100 μ L of Standard or activated sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.
3. 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 one more time for a **total of two 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 **two times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). 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.*

4. Dispense two (2) drops or 100 μ L biotin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

5. Repeat wash procedure as described in Step 3. Wash plate **five times**.
6. Dispense two (2) drops or 100 μL avidin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.
7. Repeat wash procedure as described in Step 5.
8. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
9. Add 100 μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
10. Add 100 μL Stop Solution to each well. Mix well.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-1 α 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-1 α 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 (0 pg/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of IL-1 α 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-1 α concentration.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

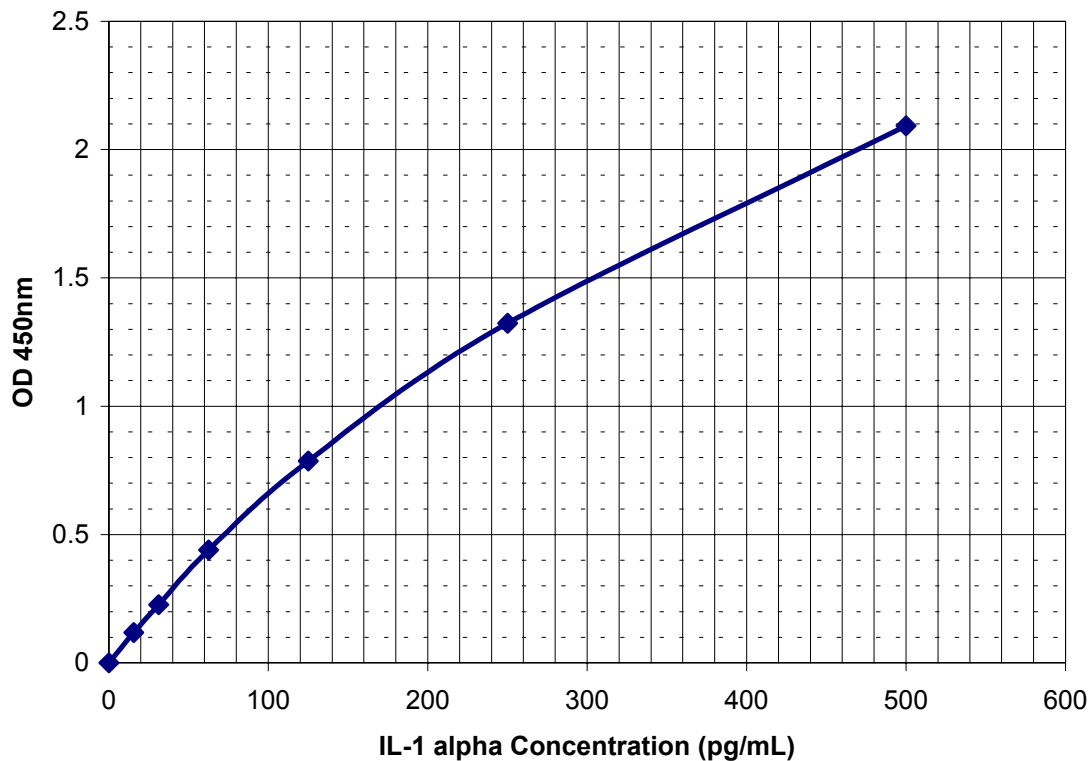
TYPICAL DATA

Results of a typical standard run of a IL-1 α 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 unknowns. Each user should obtain their own standard curve.

EXAMPLE ONE

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

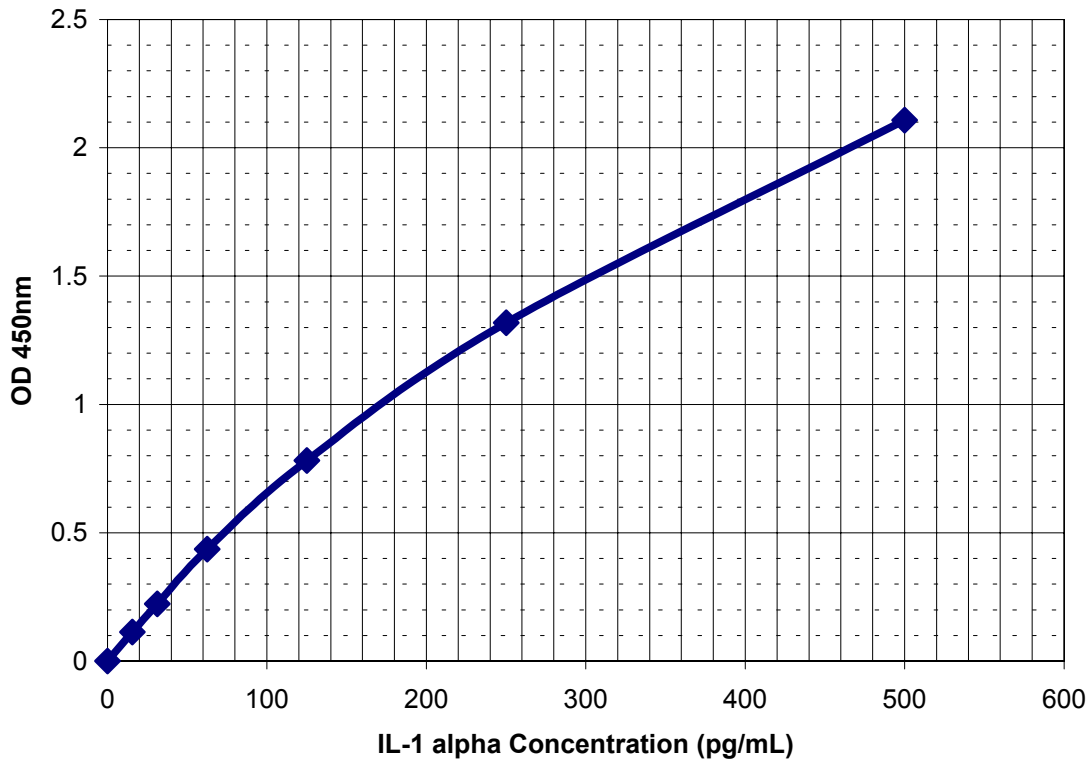
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.060, 0.058	0.059	0
15.6	0.177, 0.176	0.177	0.118
31.3	0.282, 0.290	0.286	0.227
62.5	0.507, 0.491	0.499	0.440
125	0.848, 0.843	0.846	0.787
250	1.399, 1.366	1.383	1.323
500	2.146, 2.156	2.151	2.092



EXAMPLE TWO

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

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.061, 0.063	0.062	0
15.6	0.176, 0.175	0.176	0.114
31.3	0.282, 0.288	0.285	0.223
62.5	0.491, 0.504	0.498	0.436
125	0.837, 0.848	0.843	0.781
250	1.357, 1.405	1.381	1.319
500	2.130, 2.208	2.169	2.107



PERFORMANCE CHARACTERISTICS (Quantikine)

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	Calibrator Diluent I assay			Calibrator Diluent II assay		
	1	2	3	1	2	3
n	16	16	16	16	16	16
Mean (pg/mL)	30	100	300	30	100	300
Standard Deviation (pg/mL)	1.0	3.1	10.2	1.1	3.5	8.9
Coefficient of Variation (%)	3.3	3.1	3.4	3.7	3.5	3.0

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 16 different assays.

Sample	Calibrator Diluent I assay			Calibrator Diluent II assay		
	1	2	3	1	2	3
n	16	16	16	16	16	16
Mean (pg/mL)	30	100	300	30	100	300
Standard Deviation (pg/mL)	1.6	4.4	12.3	1.9	4.8	12.9
Coefficient of Variation (%)	5.3	4.4	4.1	6.3	4.8	4.3

3. RECOVERY

The recovery of IL-1 α spiked to levels throughout the range of the assay followed by activation in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	98	80-109%
Serum	108	78-120%
EDTA plasma (platelet-poor)	99	85-123%

4. SENSITIVITY

The minimum detectable dose of IL-1 α 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 using a standard curve generated with Calibrator Diluent I is 5.0 pg/mL and using Calibrator Diluent II is 4.6 pg/mL.

5. SPECIFICITY

This sandwich ELISA recognizes both natural and recombinant human IL-1 α . The factors listed below were prepared at 50 ng/mL. In Calibrator Diluent I and Calibrator Diluent II and assayed for cross-reactivity. Preparations of the following

factors at 50 ng/mL. In a mid-range rh IL-1 α Control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human				Recombinant Mouse	
IL-1 α	ANG	IGF-I	SLP1	IL-1 α	bFGF acidic
IL-1 β	CNTF	LIF	TNF- α	IL-1 β	bFGF basic
IL-1 ra	β -ECGF	M-CSF	TNF- β	IL-3	mEGF
IL-2	EGF	MCP-1	sTNF RI	IL-4	
IL-3	EPO	MIC-1 α	sTNF RII	IL-5	
IL-4	FGF-basic	MIP-1 β	VEGF	IL-7	
IL-5	FGF-acidic	β -NGF		IL-9	
IL-6	FGF-5	OSM		IL-10	
IL-6 sR	FGF-6	PDGF-AA		EGF	
IL-7	G-CSF	PDGF-AB		GM-CSF	
IL-8	GRO- α	PDGF-BB		LIF	
IL-9	HB-EGF	PTN		MIP-1 β	
IL-10	HGF	PANTES		SCF	
IL-11	IFN- γ	SCF		TNF- α	

Recombinant human IL-1 sRI does not cross-react in this assay. However, interference was observed at concentrations greater than 10,000 pg/mL.

Recombinant human IL-1 sRII does not cross-react in this assay. Minimal interference was observed at levels equal to or greater than 30,000 pg/mL, which is above normal levels.

6. CALIBRATION

This immunoassay is calibrated against NIBSC Standard (Reference preparation) Code No. 86/632.

7. SAMPLE VALUES

Serum - Fifty serum samples were evaluated in this assay and all had levels which fell below the lowest IL-1 α standard, 15.6 pg/mL.

Cell culture supernatant - Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate and stimulated with 10 μ g/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of natural IL-1 α .

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	87	20
Stimulated	1325	400

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