

Quantikine[®]

Human IL-1 α /IL-1F1 Immunoassay

Catalog Number DLA50

SLA50

PDLA50

For the quantitative determination of human interleukin 1 alpha (IL-1 α) concentrations in cell culture supernates, 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

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 seen. For reviews on the properties and activities of IL-1 α and IL-1 β , see references 1 - 3.

IL-1 α and IL-1 β are structurally related polypeptides that show approximately 25% homology at the amino acid level (2). Both are synthesized as 31 kDa precursors that are subsequently cleaved into proteins with molecular weights of approximately 17.5 kDa (4, 5). Neither IL-1 α nor IL-1 β contains a typical hydrophobic signal peptide sequence (6 - 8), but evidence suggests that these factors can be secreted by non-classical pathways (9, 10). A large proportion of IL-1 α is retained intracellularly in its precursor form (3). A portion of this unprocessed IL-1 α is transported to the cell surface and remains associated with the cell membrane (1, 3, 11). The membrane-bound, unprocessed IL-1 α is apparently biologically active, acting in a paracrine fashion on adjacent cells having IL-1 receptors (1, 3). 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 (10 - 13). Intracellular IL-1 β consists exclusively of the 31 kDa precursor form (5). 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 (5, 9, 10, 14). The specific protease apparently responsible for the processing of IL-1 β , designated interleukin1 β -converting enzyme (ICE), has been described (14).

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 (1, 3, 15). IL-1 RI has been cloned from mouse and human cells (16) 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 (1, 3). 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 (1, 16). In general, IL-1 α binds better to the type I receptor and IL-1 β binds better to the type II receptor (1). At present, the mechanisms involved in the transduction of the signal initiated by binding of IL-1 are not well characterized (1).

IL-1 possesses a wide variety of biological activities. It has been shown to induce prostaglandin synthesis in endothelial cells and smooth muscle cells (17, 18). In the liver, IL-1 initiates the acute phase response resulting in an increase in hepatic protein synthesis and decreased albumin production (19). IL-1 induces collagenase production in synovial cells and cartilage and calcium resorption in bones (20, 21). 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 (22 - 24). IL-1 also effects the

endocrine system, acting directly on the adrenal glands to induce steroidogenesis (25). In small doses, IL-1 induces insulin production, but in larger doses is cytotoxic to β cells of the pancreas (26). It has been shown to be a hypoglycemic agent in normal mice and genetically altered, insulin-resistant mice (27). 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 (28, 29). It activates T cells, resulting in IL-2 production and expression of IL-2 receptors (30). IL-1 also induces the production of GM-CSF and IL-4 from activated T cells (31). It induces B cell proliferation and maturation and increased immunoglobulin synthesis (32, 33). IL-1, in synergy with other cytokines, plays a role in NK cell activation and LAK production, resulting in tumoricidal activity (34, 35).

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 including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, and atherosclerosis (1, 3).

The Quantikine Human IL-1 α Immunoassay is a 3.5 - 4.5 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 *E. coli*-expressed Quantikine kit standards. These results indicate that the Quantikine Human IL-1 α Immunoassay kit can be used to determine relative mass values for natural IL-1 α .

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-1 α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1 α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-1 α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1 α 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.
- 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.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # DLA50	Cat. # SLA50
IL-1α Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-1 α .	890036	1 plate	6 plates
IL-1α Conjugate - 21 mL/vial of polyclonal antibody against IL-1 α conjugated to horseradish peroxidase with preservatives.	890037	1 vial	6 vials
IL-1α Standard - 1.25 ng/vial of recombinant human IL-1 α in a buffered protein base with preservatives; lyophilized.	890038	1 vial	6 vials
Assay Diluent RD1-83 - 11 mL/vial of a buffered protein base with preservatives.	895875	1 vial	3 vials
Calibrator Diluent RD5-5 - 21 mL/vial of a buffered protein base with preservatives. <i>For cell culture supernate samples.</i>	895485	1 vial	6 vials
Calibrator Diluent RD6C - 21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</i>	895015	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	—	4 strips	24 strips

DLA50 contains sufficient materials to run an ELISA on one 96 well plate.

SLA50 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDLA50). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Calibrator Diluent RD5-5	
	Calibrator Diluent RD6C	
	Assay Diluent RD1-83	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Microplate Wells	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.*	

*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.
- Human IL-1 α Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Calibrator Diluent RD6C contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

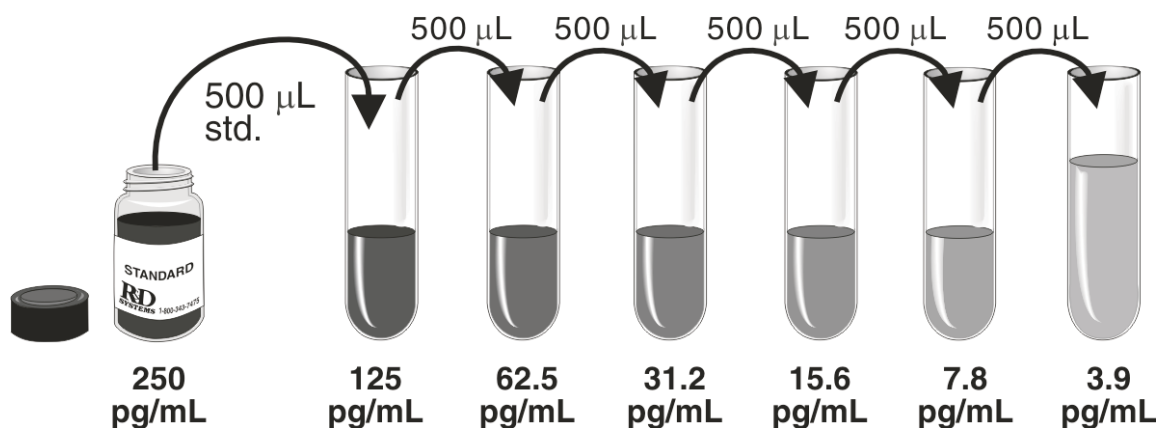
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 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.

IL-1 α Standard - Reconstitute the IL-1 α Standard with 5 mL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6C (*for serum/plasma samples*). This reconstitution produces a stock solution of 250 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 μL of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (250 pg/mL). The appropriate Calibrator Diluent 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 samples, standards, and controls 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, reseal.
3. Add 50 μL of Assay Diluent RD1-83 to each well. Assay Diluent RD1-83 may contain a precipitate. Mix well before and during its use.
4. Add 200 μL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided as a record of samples and standards assayed.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 toweling.
6. Add 200 μL of IL-1 α Conjugate to each well. Cover with a new adhesive strip.
For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
For Serum/Plasma Samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate 20 minutes at room temperature.
Protect from light.
9. 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. 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.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents and standards as instructed.



2. Add 50 μL of Assay Diluent RD1-83 to each well.



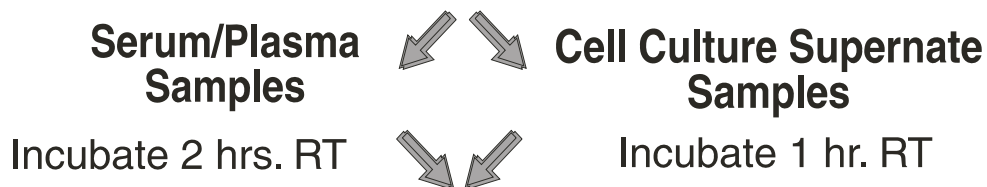
3. Add 200 μL Standard, sample, or control to each well. Incubate 2 hrs. RT.



4. Aspirate and wash 3 times.



5. Add 200 μL Conjugate to each well.



6. Aspirate and wash 3 times.



7. Add 200 μL Substrate Solution to each well. Incubate 20 min. RT.

Protect from light.



8. Add 50 μL Stop Solution to each well.
Read at 450 nm within 30 min.

λ correction 540 or 570 nm

CALCULATION OF RESULTS

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

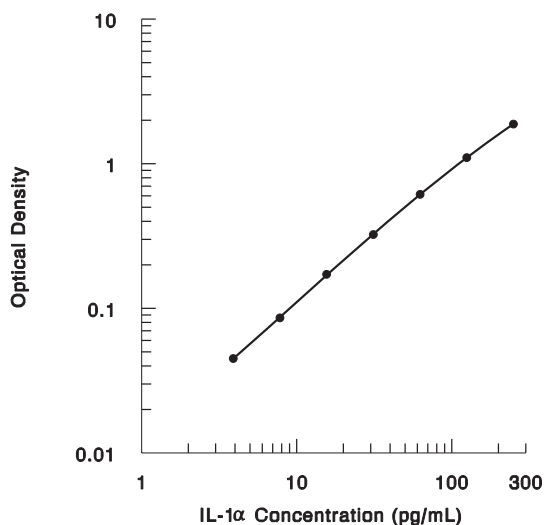
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-1 α 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 concentrations read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

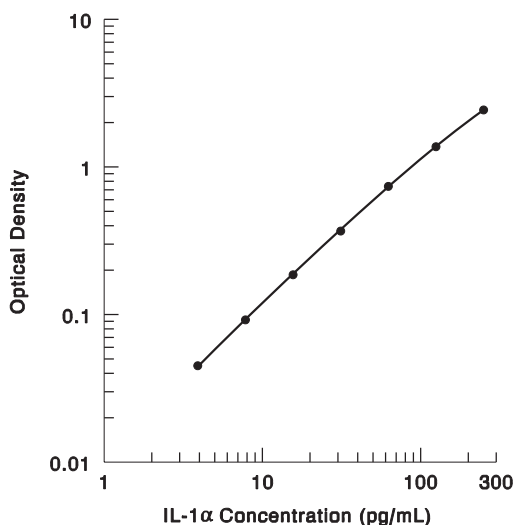
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5-5



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.015 0.057	0.014	—
3.9	0.060 0.099	0.059	0.045
7.8	0.100 0.185	0.100	0.086
15.6	0.187 0.332	0.186	0.172
31.2	0.343 0.612	0.338	0.324
62.5	0.644 1.111	0.628	0.614
125	1.121 1.865	1.116	1.102
250	1.924	1.895	1.881

Calibrator Diluent RD6C



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.021 0.064	0.019	—
3.9	0.064 0.113	0.064	0.045
7.8	0.109 0.200	0.111	0.092
15.6	0.210 0.376	0.205	0.186
31.2	0.398 0.762	0.387	0.368
62.5	0.754 1.392	0.758	0.739
125	1.387 2.415	1.390	1.371
250	2.489	2.452	2.433

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 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.

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.

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	28.6	125	185	27.9	123	187
Standard deviation	0.4	1.4	4.0	1.2	4.7	6.3
CV (%)	1.4	1.1	2.2	4.3	3.8	3.4

Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	26.9	122	191	20.5	60.0	121
Standard deviation	0.4	4.6	6.6	1.7	3.1	5.2
CV (%)	1.5	3.8	3.5	8.3	5.2	4.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=6)	107	74 - 127%
EDTA plasma (n=6)	104	84 - 121%
Citrate plasma (n=6)	97	71 - 119%
Heparin plasma (n=6)	104	72 - 126%
Cell culture media (n=4)	96	91 - 104%

SENSITIVITY

The minimum detectable dose of IL-1 α is typically less than 1.0 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1 α produced at R&D Systems. The NIBSC/WHO 1st International recombinant human IL-1 α Standard 86/632 was evaluated in this kit.

The dose response curve of the NIBSC standard 86/632 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human IL-1 α kit to equivalent NIBSC International Units, use the equation below:

$$\text{NIBSC (86/632) equivalent value (IU/mL)} = 0.153 \times \text{Quantikine Human IL-1}\alpha \text{ value (pg/mL)}$$

LINEARITY

To assess linearity of the assay, the following biological samples spiked with high concentrations of IL-1 α were diluted with the appropriate Calibrator Diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	% $\frac{\text{Observed}}{\text{Expected}}$
Cell culture media	neat	141		
	1:2	68.3	70.5	97
	1:4	34.9	35.3	99
	1:8	16.2	17.6	92
Serum	neat	273		
	1:2	124	136	91
	1:4	62	68	91
	1:8	31	34	91
	1:16	18	17	106
EDTA plasma	neat	298		
	1:2	144	149	97
	1:4	66	74	89
	1:8	34	37	92
	1:16	18	18	100
Heparin plasma	neat	248		
	1:2	130	124	105
	1:4	59	62	95
	1:8	27	31	87
	1:16	15	16	94
Citrate plasma	neat	203		
	1:2	103	102	101
	1:4	52	51	102
	1:8	23	26	88
	1:16	12	13	92

SAMPLE VALUES

Serum - Forty serum samples were evaluated in this assay and all had levels which fell below the lowest IL-1 α standard, 3.9 pg/mL.

Cell Culture Supernates - 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	85	23
Stimulated	1330	395

SPECIFICITY

This assay recognizes both natural and recombinant human IL-1 α . The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range rhIL-1 α control were assayed for interference. With the exception of IL-1 sRI interference, no significant cross-reactivity or interference was observed.

Recombinant human:

ANG IL-1ra
 AR IL-2
 CNTF IL-3
 β -ECGF IL-3R α
 EGF IL-3R β
 Epo IL-4
 FGF acidic IL-5
 FGF basic IL-5R α
 FGF-4 IL-6
 FGF-5 IL-6 sR
 FGF-6 IL-7
 FGF-7 IL-8
 G-CSF IL-9
 sgp130 IL-10
 GRO α IL-11
 GRO γ IL-12
 HB-EGF IL-13
 HGF IL-18
 IFN- γ LAP
 IGF-I LIF
 IGF-II M-CSF
 IL-1 β MCP-1

MIP-1 α

MIP-1 β
 β -NGF
 OSM
 PD-ECGF
 PDGF-AA
 PDGF-AB
 PDGF-BB
 PTN
 RANTES
 SCF
 SLPI
 TGF- α
 TGF- β 1
 TGF- β 3
 TGF- β 5
 TNF- β
 sTNF RI
 sTNF RII
 VEGF

Recombinant mouse:

EGF
 GM-CSF
 IL-1 α
 IL-1 β
 IL-3
 IL-4
 IL-5
 IL-6
 IL-7
 IL-9
 IL-13
 LIF
 MIP-1 α
 MIP-1 β
 SCF
 TNF- α

Recombinant chicken:

TGF- β 3

Other proteins:

bovine FGF basic
 porcine PDGF
 porcine TGF- β 1

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.

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

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