Human IL-1α ELISA Kit

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

Catalogue Number: EL10040

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca
Secure Online Store: www.anogen.net

S7.5 (03) IL-1α

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INTENDED USE

This Human IL-1α ELISA kit is to be used for the *in vitro* quantitative determination of human Interleukin-1 Alpha (IL-1α) 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

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 31 kDa precursor that lacks a signal peptide. Cleavage of the precursor is via the cysteine protease calpain, resulting in a 17.5 kDa mature IL-1 molecule. Being active in the processed form, the IL-1 precursor is also biologically active via specific cell binding. A portion of the precursor is transported to the cell surface and associated with the cell membrane. Precursor IL-1α can be released and cleaved by extracellular proteases when the cells die, and can also be cleaved by activation of the calcium-dependent, membrane-associated calpains. Nearly all microbes and microbial products induce the production of IL-1α. Furthermore, IL-1α can be produced in monocytes and other cells in the 31 kDa precursor state.

IL-1α can act on macrophages or monocytes by inducing 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 supressed 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.

This IL-1α *ELISA Kit is a ready-to-use 3.5-hour solid phase immunoassay readily capable of measuring 0 to 500 pg/mL in cell culture supernatant, serum and plasma. However, data collection for proliferation testing for* IL-1α *detection will require at least 3-4 days for completion. This assay has shown specific reaction with* IL-1α*, and no cross-reactivity with various other cytokine superfamily proteins.*

PRINCIPLE OF THE ASSAY

This IL-1α enzyme linked immunosorbent assay (ELISA) applies a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-1α. Standards or samples are then added to the appropriate microtiter plate wells and incubated. After washing to remove unbound IL-1α and other components of the sample, biotin-conjugated polyclonal antibody specific to IL-1α is added and incubated. If present, IL-1α will bind and become immobilized by the antibody pre-coated on the wells and then become "sandwiched" by the biotin conjugate. In order to quantitatively determine the amount of IL-1α 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, each having 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-1α, 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 ± 2 nm.

In order to measure the concentration of IL-1α in the samples, the reagents of this kit include 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 in conjunction with 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 IL-1α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- The Human IL-1α ELISA kit is not for use in clinical diagnostic procedures, and is for laboratory use only.
- Although all manufacturing precautions have been exercised to ensure that this product will be suitable for use with all validated sample types as designated in the product insert, the possibility of interference cannot be excluded due to the variety of proteins that may exist within the sample.
- The Calibrator Diluent selected for the standard curve should be consistent with the assay samples. If the values generated by the samples are greater than the uppermost standard, the samples dilution should be adjusted with the appropriate Calibrator Diluent and the assay should be repeated.

REAGENTS PROVIDED

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

	96 tests

1. **IL-1α MICROTITER PLATE** (Part EL40-1) ***96 wells***
   Pre-coated with anti-human IL-1α monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL40-2) ***6 mL***
   Anti-human IL-1α polyclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL40-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-1α STANDARD** (Part EL40-4) ***2 vials***
   Recombinant human IL-1α (2000 pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL40-5) ***25 mL***
   Animal protein with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL40-6) ***25 mL***
   Cell culture medium with animal protein and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL40-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    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 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. 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 stored at ≤-20°C. Avoid repeated freeze-thaw cycles. Special caution: The supernatant may contain a certain level of latent IL-1α 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-1α.

b) **Serum**: Use a serum separator tube (SST) and allow samples to clot for one hour at room temperature. For complete release of IL-1α, incubate overnight at 2-8 °C before centrifugation. Centrifuge for 10 minutes at 1000 x g (4°C). Remove serum and assay (see activation procedure) immediately or aliquot and store at ≤-20°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°C is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at ≤-70°C. Avoid repeated freeze-thaw cycles.

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):** 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°C. Mix well before use.

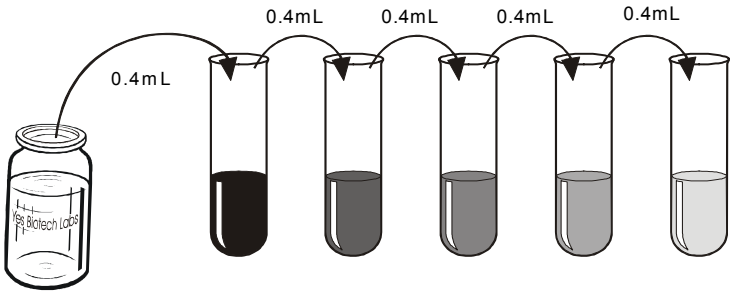
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

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-1α Standard:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-1α 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-1α 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-1α Standard will serve as the **high standard (500 pg/mL)** and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



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

ASSAY PROCEDURE

1. Prepare Wash Buffer and 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 and Samples to the Microtiter Plate.*

Wells	Contents			**Wells**	Contents		
1A, 1B	Standard 1	**0 pg/mL**	(S1)	**2A, 2B**	Standard 5	**125 pg/mL**	(S5)
1C, 1D	Standard 2	**15.6 pg/mL**	(S2)	**2C, 2D**	Standard 6	**250 pg/mL**	(S6)
1E, 1F	Standard 3	**31.3 pg/mL**	(S3)	**2E, 2F**	Standard 7	**500 pg/mL**	(S7)
1G, 1H	Standard 4	**62.5 pg/mL**	(S4)	**2G,2H**	**IL-1α** 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 100μL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL IL- IL-1α Biotin conjugate to each wells. 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 350 μL/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 Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

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

10. 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-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 (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-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.

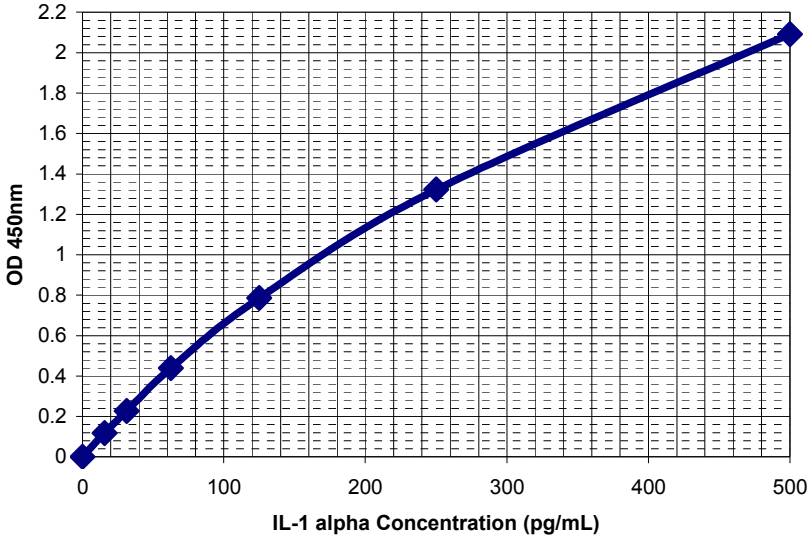
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

TYPICAL DATA

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

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

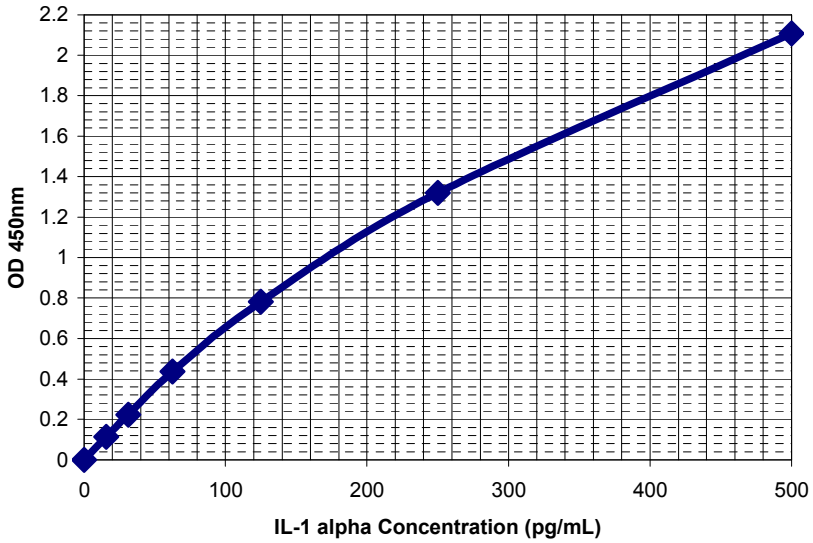
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.060, 0.058	0.059	0
15.6	0.177, 0.176	0.177	0.118
31.3	0.282, 0.290	0.286	0.227
62.5	0.507, 0.491	0.499	0.440
125	0.848, 0.843	0.846	0.787
250	1.399, 1.366	1.383	1.323
500	2.146, 2.156	2.151	2.092



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

1. INTRA-ASSAY PRECISION

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

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**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.

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**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

By employing various samples, the recovery of IL-1α was evaluated at different amounts of IL-1α throughout the range of the assay. All samples were mixed and assayed in duplicate.

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 quantities of human IL-1α as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 5.0 pg/mL and 4.6 pg/mL respectively. The two standard deviations above the mean optical density of the 16 replicates of the zero standard were defined as the minimum detectable quantities.

5. SPECIFICITY

This sandwich ELISA can detect both natural and recombinant human IL-1α. The following factors 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 recombinant human IL-1α Control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human				Recombinant Mouse	
IL-1α	ANG	IGF-I	SLP1	IL-1α	bFGF acidic
IL-1β	CNTF	LIF	TNF-α	IL-1β	bFGF basic
IL-1 rα	β-ECGF	M-CSF	TNF-β	IL-3	mEGF
IL-2	EGF	MCP-1	sTNF RI	IL-4	
IL-3	EPO	MIC-1α	sTNF RII	IL-5	
IL-4	FGF-basic	MIP-1β	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-1β	
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. **EXPECTED NORMAL VALUES**
   Serum - Fifty serum samples were evaluated in this assay and all had levels which fell below the lowest IL-1α standard, 15.6 pg/mL.

   Cell culture supernatant - Mononuclear cells (5 x 10^6 cells/mL) of human peripheral blood were stimulated with 10 μg/mL PHA in a culture with RPMI 1640 medium supplemented with 5% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Aliquot the culture supernate and assay the IL-1α quantity on days 1 and 5.

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

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