Human IL-1β ELISA Kit

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

Catalogue Number: EL10028

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03/14-04) IL-1β

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INTENDED USE

This Human IL-1β ELISA kit is to be used for the *in vitro* quantitative determination of human interleukin-1β (IL-1β) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. 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 considered the first of the super-family of regulatory and inflammatory cytokines. There are two distinct IL-1 proteins: Interleukin 1α and Interleukin 1β. The two forms of IL-1 are distinct gene products; they recognize the same receptor and share biological properties. IL-1 has a number of alternative names, including lymphocyte activating factor, endogenous pyrogen, catabolin, hemopoietin-1, melanoma growth inhibition factor, and osteoclast activating factor. The properties and biological activities of IL-1 have been extensively reviewed. IL-1 is expressed by many cells and has multiple functions including local inflammation. Cells known to express IL-1β include astrocytes, adrenal cortical cells, natural killer (NK) cells, macrophages and monocytes, endothelial cells, keratinocytes, megakaryocytes and platelets, neurons, neutrophils, oligodendroglia, osteoblasts, Schwann cells, trophoblasts, and T cells plus fibroblasts.

The biological properties of IL-1 show some overlap with other cytokines including tumor necrosis factor (TNF) and interleukin 6 (IL-6), which are all capable of stimulating T and B lymphocytes, augmenting cell proliferation and initiating or suppressing gene expression for several proteins. Although IL-1 is generally thought of as a prototypical pro-inflammatory cytokine, the effects of IL-1 are not limited to inflammation. Following bacterial or immunoglobulin ligation of monocyte/macrophage CD14 (the LPS receptor) or CD64 (the IgG receptor), IL-1 can be released into a local environment. Within this environment, IL-1 impacts a number of cells. First, capillary endothelial cells are induced to do two things, one, secrete chemokines such as MCP-1, and two, up-regulate the expression of vascular adhesion molecules such as E-Selectin, ICAM-1 and VCAM-1. MCP-1 provides a stimulus for chemotaxis and activates mononuclear cell integrins, thus facilitating mononuclear infiltration into an area of early inflammation. IL-1 also induces expression of itself in newly arriving monocytes, thus reinforcing the overall process. In terms of other pro-inflammatory molecules, IL-1 apparently is needed for the efficient production of IFN-γ. On resident NK cells, IL-1 apparently works in conjunction with macrophage-derived IL-12 to induce IFN-γ secretion, resulting in an IFN-γ induced activation of macrophages. Finally, IL-1 also induces the expression of MMPs from resident fibroblasts. This function can have at least two effects: first extracellular matrix degradation can facilitate monocyte migration, and second, MMPs are known to degrade IL-1β, thus down-modulating the local inflammatory response initiated by IL-1.

This IL-1β ELISA is a ready-to-use 3.5-hour solid phase immunoassay capable of measuring IL-1β levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 400 pg/mL. This assay has shown no cross-reactivity with various other

cytokines super-family proteins, and is expected to be used effectively for further investigations into the relationship between IL-1β and various diseases.

PRINCIPLE OF THE ASSAY

This IL-1β 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-1β. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated monoclonal antibody preparation specific for IL-1β and incubated. IL-1β, if present, will bind and become immobilized by the antibody pre-coated on the wells and then become "sandwiched" by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound IL-1β and other components of the sample. In order to quantitatively determine the amount of IL-1β 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-1β, 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-1β 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 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-1β concentration (pg/mL). The concentration of IL-1β in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- The Human IL-1β ELISA kit is for laboratory research use only, and is not intended for use in clinical diagnostic procedures. 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-1β MICROTITER PLATE** (Part EL28-1) ***96 wells***
   Pre-coated with anti-human IL-1β monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL28-2) ***6 mL***
   Anti-human IL-1β monoclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL28-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-1β STANDARD** (Part EL28-4) ***2 vials***
   Recombinant human IL-1β (800pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL28-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL28-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

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

8. **TMB SUBSTRATE B** (Part 30010) ***11 mL***
   Ready to use.

9. **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 60 min. 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. 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:** 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.
c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulates.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, cell culture supernatant, and urine samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- 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.

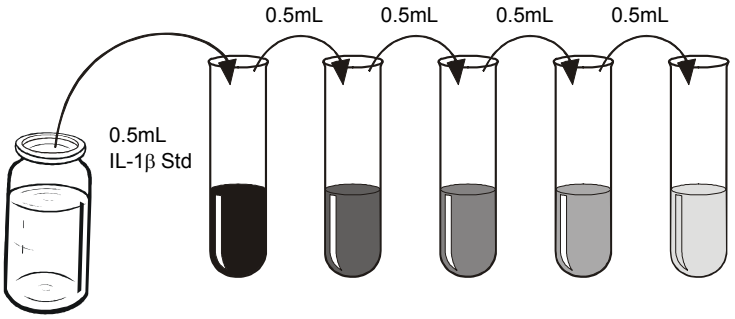
1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **IL-1β Standard:**

   a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-1β Standard with either

2.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 400 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-1β standard stock solution can be stored frozen (-20°C) 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 (12.5 pg/mL to 400 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-1β Standard will serve as the **high standard (400 pg/mL**) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-1β Standard 400 pg/ml	200 pg/ml	100 pg/ml	50 pg/ml	25 pg/ml	12.5 pg/ml

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-1β 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	0 **pg/mL** (S1)	**2A, 2B**	Standard 5	100 **pg/mL** (S5)
1C, 1D	Standard 2	12.5 **pg/mL** (S2)	**2C, 2D**	Standard 6	200 **pg/mL** (S6)
1E, 1F	Standard 3	25 **pg/mL** (S3)	**2E, 2F**	Standard 7	400 **pg/mL** (S7)
1G, 1H	Standard 4	50 **pg/mL** (S4)	**2G, 12H**	IL-1β 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 37°C.

3. Without discarding the standards and samples, add 50μL IL-1β Biotin conjugate to each well. Mix well. Cover and incubate for 1 hour at 37°C.

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 37°C.

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 TMB Substrate to each well. Cover and incubate for 15 minutes at 37°C.

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-1β 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-1β 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) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-1β 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-1β 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.

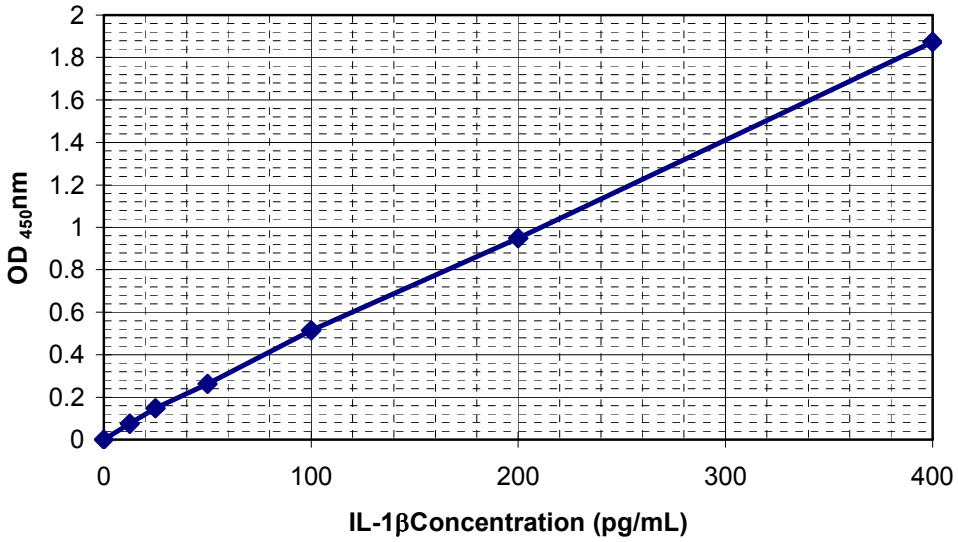
TYPICAL DATA

Results of a typical standard run of a IL-1β 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.

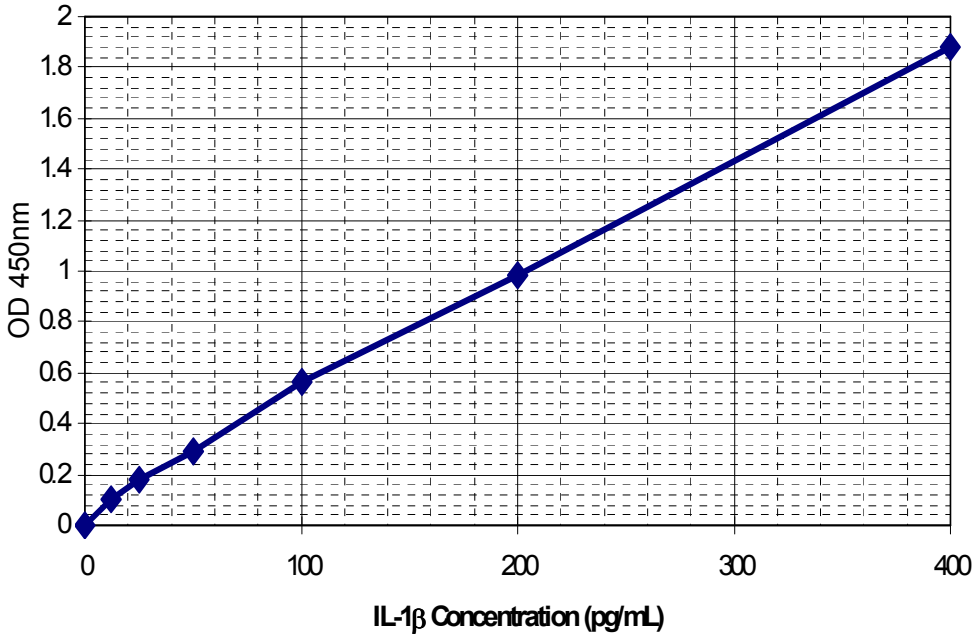
Standard (pg/mL)	**O.D. (450 nm)**	**Mean**	**Zero Standard Subtracted** (Std.)-(S1)
0	0.080, 0.079	0.080	0
12.5	0.153, 0.157	0.155	0.075
25	0.214, 0.242	0.228	0.148
50	0.313, 0.371	0.342	0.262
100	0.587, 0.601	0.594	0.514
200	1.022, 1.034	1.028	0.948
400	1.930, 1.978	1.954	1.874



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	0.081, 0.079	0.080	0
12.5	0.191, 0.179	0.185	0.105
25	0.268, 0.254	0.261	0.181
50	0.378, 0.364	0.371	0.291
100	0.645, 0.639	0.642	0.562
200	1.075, 1.058	1.067	0.987
400	1,929, 1.986	1.958	1.878



PERFORMANCE CHARACTERISTICS

1. I**NTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by replicates of 20 in 1 assay.

	Calibrator Diluent I assay			***Calibrator Diluent II assay***		
Sample	**1**	**2**	***3***	**1**	**2**	**3**
N	20	20	20	20	20	20
Mean (pg/mL)	42	175	340	30	141	298
Standard Deviation (pg/mL)	3.3	10.7	20.1	2.2	7.8	18.2
Coefficient of Variation (%)	*7.9*	*6.1*	*5.9*	*7.3*	*5.5*	*6.1*

2. **INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of known concentration were assayed by replicates on 20 different assays.

	Calibrator Diluent I assay			***Calibrator Diluent II assay***		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	37	152	322	49	146	315
Standard Deviation (pg/mL)	2.6	8.1	18.4	3.6	9.3	16.1
Coefficient of Variation (%)	*7.0*	*5.3*	*5.7*	*7.3*	*6.4*	*5.1*

3. **RECOVERY**

By employing various samples, the recovery of IL-1β was evaluated in 3 different amounts of IL-1β throughout the range of the assay.

Sample Type	**Average recovery %**	**Range %**
Serum	98	89 - 105
Cell culture media	99	87 - 106
Plasma	87	80 - 104

4. **SENSITIVITY**

The minimum detectable quantities of human IL-1β as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 2.0 pg/mL and 2.0 pg/mL respectively. The two standard deviations above the mean optical density of the 20 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-1β. This kit exhibits no detectable cross-reactivity with human; IL-1α, IL-1RA, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IFN-α, IFN-γ, TNF-α or mouse IL-1β.

6. **CALIBRATION**

This immunoassay is calibrated against WHO Standard Code No.: 86/552.

7. **EXPECTED NORMAL VALUES**

Biological samples from apparently healthy, normal individuals were collected and the IL-1β concentration was measured. Serum / plasma samples (n=20) averaged less than 10 pg/mL while Urine sample (n=20) averaged less than 10 pg/mL.

REFERENCES

1. Bazan, J.F., et al. (1996) Nature 379:591.
2. Billiau, A, (1996) Adv. Immunol. 62:61.
3. da Cunha. A., et al. (1993) J. Neuroimmunol.42:71.
4. Dayer-J.M. (1997) Immunologist 56:192.
5. Dianrello, C. A., et al. (1987)J. Immunol.139:1902.
6. Dinarello, C.A.(1994) Eur. Cytokine Netw. 5:517.
7. Dodds, R. A., et al. (1994) J. Histochem. Cytochem. 42:733.
8. Freidin, M., et al. (1992) Proc, Natl. Acad. Sci, USA 89:10440.
9. Gonzales Hermandez, J. A., et al. (1996) Clin. Exp. Immunol. 99:137.
10. Hunter, C.A.(1996) J. Immunol. 155:4347.
11. Jiang, S., et al. (1994) Blood 84:4151.
12. J. Immunol 144: 3034.
13. Jokhi, P.P., et al. (1997) Cytokine 9:126.
14. Kupper, T.S. &R.W. Groves (1995) J. Invest. Dermatol. 105: 62S.
15. Kusano, K., et al.(1994) Endocrinology 139:1540.
16. Loppnow, H., et al. (1998) Blood 91:134.
17. Nalyak, M., et al. (1994) J. Clin. Immunol. 14:20.
18. Nockher, W.A. & J. Scherberich (1997) J. Immunol. 158:1345.
19. Nylander Lundquist, E & T Egelrud (1997) Eur. J. Immunol. 27:2165.
20. Rollins, B.J. (1997) Blood 90:909.
21. Skundric, D.S., et al.(1997) J. Neuroimmunol. 74:9.
22. Sporri, B., et al (1996) Cytokine8:63.
23. van de Winkel J. G.J. & P.J. A. Capel (1993) Immunol. Today 14:215.
24. Warner, S.J.C., et al. (1987) J. Immunol. 139:1911.
25. Wewers, M.D., et al. (1987) J. Immunol 159:5964.