# Quantikine® ELISA

## Human IL-1α/IL-1F1 Immunoassay

Catalog Number DLA50
SLA50
PDLA50

For the quantitative determination of human Interleukin 1 alpha (IL-1α) 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-1α and IL-1β, 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-1α and IL-1β, see references 1-3.

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid (aa) 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-1α nor IL-1β 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-1α is retained intracellularly in its precursor form (3). A portion of this unprocessed IL-1α is transported to the cell surface and remains associated with the cell membrane (1, 3, 11). The membrane-bound, unprocessed IL-1α is apparently biologically active, acting in a paracrine fashion on adjacent cells having IL-1 receptors (1, 3). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the 17.5 kDa processed form (10-13). Intracellular IL-1β consists exclusively of the 31 kDa precursor form (5). Extracellular IL-1β consists of a mixture of both unprocessed and mature IL-1β. 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-1β, designated interleukin1β-converting enzyme (ICE), has been described (14).

IL-1α and IL-1β 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 aa residues, whereas the type I receptor has a cytoplasmic domain of 213 aa residues (1, 16). In general, IL-1α binds better to the type I receptor and IL-1β binds better to the type II receptor (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-1α/IL-1F1 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human IL-1α levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-1α and antibodies raised against the recombinant protein. Results obtained for natural human IL-1α showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for human IL-1α.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-1α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-1α 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-1α 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 diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

## 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.

## PRECAUTIONS

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.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | CATALOG # DLA50 | CATALOG # SLA50 | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human IL-1α Microplate** | 890036 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-1α. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Human IL-1α Standard** | 890038 | 1 vial | 6 vials | Recombinant human IL-1α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles. |
| **Human IL-1α Conjugate** | 890037 | 1 vial | 6 vials | 21 mL/vial of a polyclonal antibody specific for human IL-1α conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-83** | 895875 | 1 vial | 6 vials | 11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.* | |
| **Calibrator Diluent RD5-5** | 895485 | 1 vial | 6 vials | 21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.* | |
| **Calibrator Diluent RD6C** | 895015 | 1 vial | 6 vials | 21 mL/vial of animal serum with preservatives. *For serum/plasma samples.* | |
| **Wash Buffer Concentrate** | 895003 | 1 vial | 6 vials | 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL/vial of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

* Provided this is within the expiration date of the kit.

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. Refer to the literature accompanying your order for specific vial counts.

## 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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human IL-1α Controls (optional; R&D Systems®, Catalog # QC01-1).

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

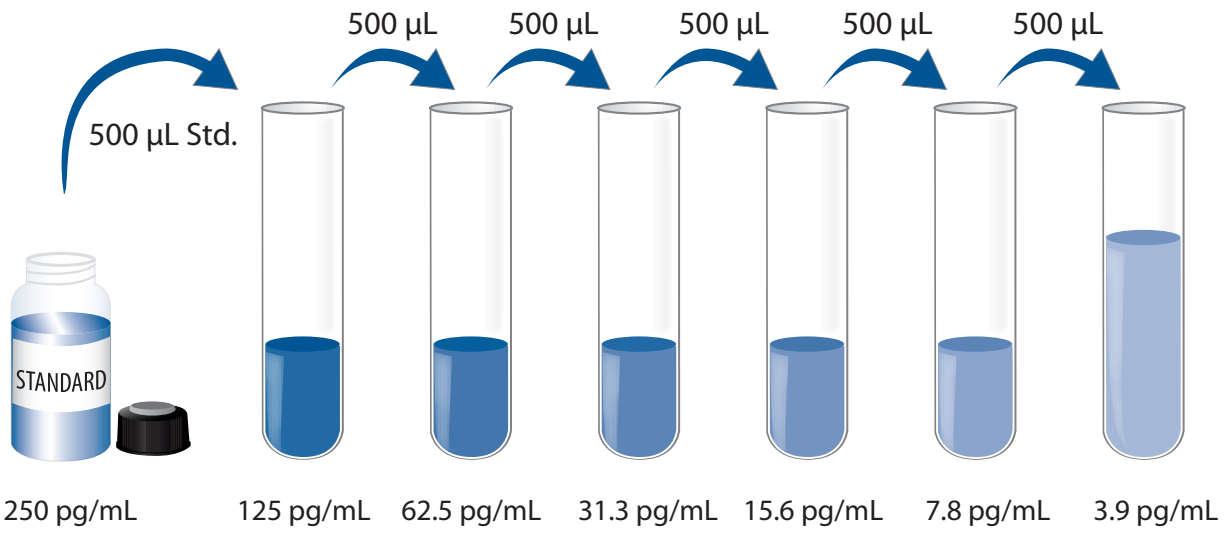
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 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.

**Human IL-1α Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-1α Standard with 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 Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6C (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-1α Standard (250 pg/mL) serves as the high standard. 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 standards, samples, 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, and 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 Human IL-1α 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.

## CALCULATION OF RESULTS

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

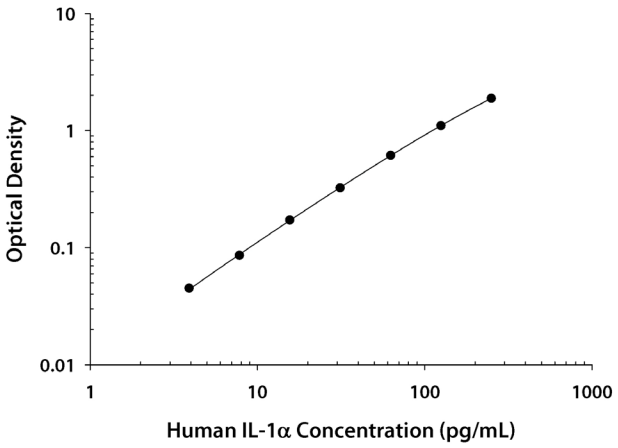
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

## TYPICAL DATA

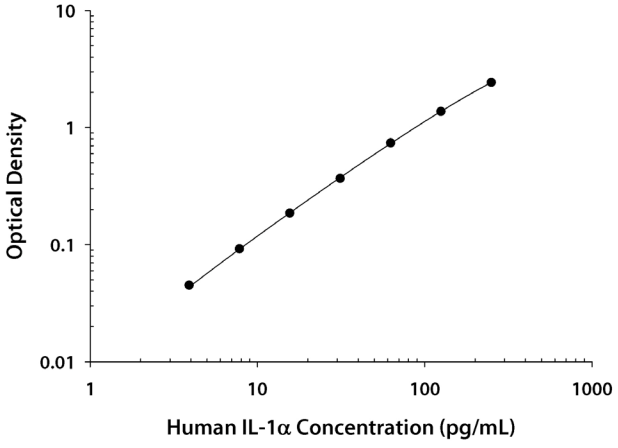
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.013<br>0.015 | 0.014 | — |
| 3.9 | 0.057<br>0.060 | 0.059 | 0.045 |
| 7.8 | 0.099<br>0.100 | 0.100 | 0.086 |
| 15.6 | 0.185<br>0.187 | 0.186 | 0.172 |
| 31.3 | 0.332<br>0.343 | 0.338 | 0.324 |
| 62.5 | 0.612<br>0.644 | 0.628 | 0.614 |
| 125 | 1.111<br>1.121 | 1.116 | 1.102 |
| 250 | 1.865<br>1.924 | 1.895 | 1.881 |

### SERUM/PLASMA ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.017<br>0.021 | 0.019 | — |
| 3.9 | 0.064<br>0.064 | 0.064 | 0.045 |
| 7.8 | 0.113<br>0.109 | 0.111 | 0.092 |
| 15.6 | 0.200<br>0.210 | 0.205 | 0.186 |
| 31.3 | 0.376<br>0.398 | 0.387 | 0.368 |
| 62.5 | 0.762<br>0.754 | 0.758 | 0.739 |
| 125 | 1.392<br>1.387 | 1.390 | 1.371 |
| 250 | 2.415<br>2.489 | 2.452 | 2.433 |

## 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. Assays were performed by at least three technicians using two lots of components.

### CELL CULTURE SUPERNATE ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **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 |

### SERUM/PLASMA ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **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 |

## RECOVERY

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

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

## SENSITIVITY

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

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of human IL-1α and diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

| Sample | Dilution | Observed (pg/mL) | Expected (pg/mL) | $\frac{\text{Observed}}{\text{Expected}} \times 100$ |
|---|---|---|---|---|
| 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 |

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1α produced at R&D Systems®. The NIBSC/WHO 1st International recombinant Human IL-1α 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-1α kit to approximate NIBSC International Units, use the equation below:

NIBSC (86/632) approximate value (IU/mL) = 0.153 x Quantikine® Human IL-1α value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma** - Forty samples from apparently healthy volunteers were evaluated for the presence of human IL-1α, and all had levels which fell below the lowest IL-1α standard, 3.9 pg/mL. No medical histories were available for the donors used in this study.

**Cell Culture Supernates** - Human peripheral blood mononuclear cells (5 x $10^6$ cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of human IL-1α.

| Condition | Day 1 (pg/mL) | Day 5 (pg/mL) |
|---|---|---|
| Unstimulated | 85 | 23 |
| Stimulated | 1330 | 395 |

## SPECIFICITY

This assay recognizes natural and recombinant human IL-1α.

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 human IL-1α control were assayed for interference. With the exception of IL-1 RI interference, no significant cross-reactivity or interference was observed.

### Recombinant human:

ANG
AR
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
gp130
GROα
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1β
IL-1ra
IL-2
IL-3
IL-3Rα
IL-3Rβ
IL-4
IL-5
IL-5Rα
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
IL-18
KGF/FGF-7
LAP
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β3
TGF-β5
TNF-β
TNF RI
TNF RII
VEGF

### Recombinant mouse:

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

### Natural proteins:

bovine FGF basic
porcine PDGF
porcine TGF-β1

Recombinant human IL-1 RI interferes in this assay at concentrations > 10,000 pg/mL.

Recombinant human IL-1 RII interferes in this assay at concentrations ≥ 30,000 pg/mL, which is above normal levels.

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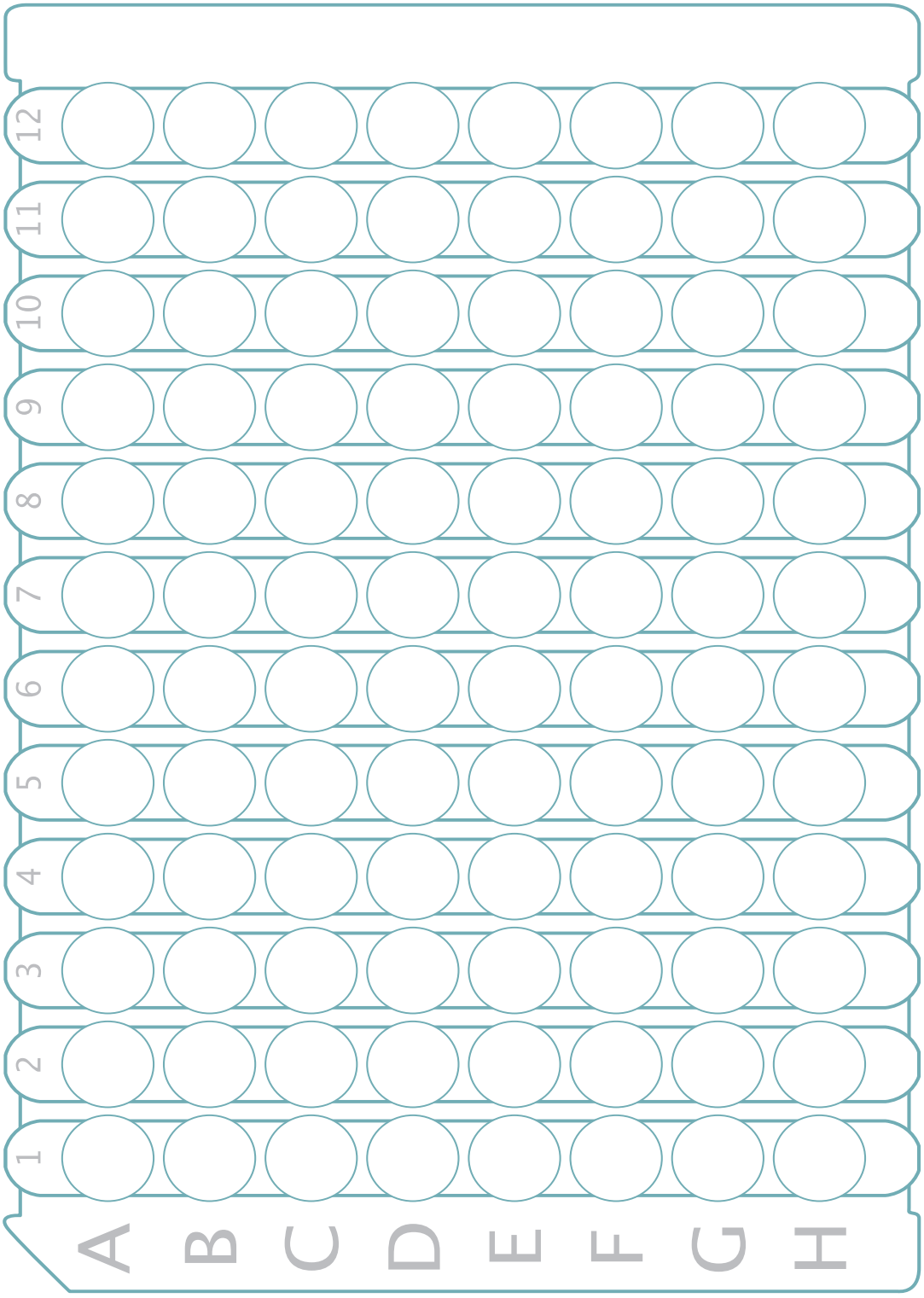
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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