Quantikine® ELISA

Human IL-1β/IL-1F2 Immunoassay

Catalog Number DLB50
SLB50
PDLB50

For the quantitative determination of human interleukin 1 beta (IL-1β) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

The Interleukin 1 (IL-1) family of proteins consists of the classic members IL-1α, IL-1β, and IL-1ra, plus IL-18, IL-33 and IL-1F5-F10. IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1). IL-1 is not produced by unstimulated cells of healthy individuals with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. However, in response to inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is observed. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (2-5).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (6, 7). Cleavage of the IL-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (9-11), but evidence suggests that these factors can be secreted by non-classical pathways (12, 13). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (14). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (13, 15). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (16, 17). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (18). 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 acids (aa), whereas the type I receptor has a 213 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (19). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (20). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (21, 22). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (23, 24). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (25).

The Quantikine Human IL-1β Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure IL-1β in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-1β and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human IL-1β accurately. Results obtained using natural IL-1β 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 natural IL-1β. Reports indicate that this and other ELISA kits calibrated using mature IL-1β as a standard will detect, but considerably underestimate, the unprocessed IL-1β precursor present in samples (26, 27). In biological samples other than cell lysates, the precursor form of IL-1β is usually not the predominant form of IL-1β present and, additionally, is not biologically active. Therefore, results obtained using this kit should provide a useful measure of the levels of active IL-1β present in biological fluids.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-1β 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-1β 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DLB50	CATALOG # SLB50	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-1β Microplate†	890039	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-1β.	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-1β Standard	890041	1 vial	6 vials	Recombinant human IL-1β 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-1β Conjugate†	890040	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human IL-1β 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	3 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use. For serum/plasma samples.*	
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.

DLB50 contains sufficient materials to run an ELISA on one 96 well plate.
SLB50 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDLB50). 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.

† This product is covered by the following U.S. patent.: 5,681,933.

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.
- Human IL-1β Controls (optional; R&D Systems, Catalog # QC01-1).

PRECAUTIONS

IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

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. Please refer to the MSDS on our website prior to use.

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

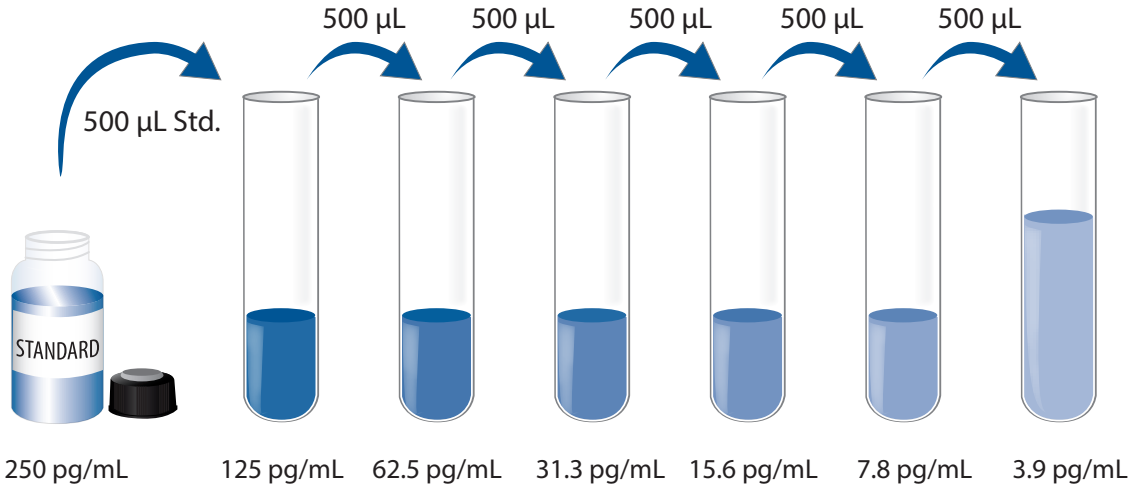
Bring all reagents to room temperature before use.

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 to 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-1β Standard - **Refer to the vial label for reconstitution volume.** - Reconstitute the Human IL-1β 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 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 Human IL-1β 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.

Note: *IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.*

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. **For Cell Culture Supernate Samples:** Proceed to step 4.
   For Serum/Plasma Samples: Add 50 µL of Assay Diluent RD1-83 to each well.
   Assay Diluent RD1-83 may contain a precipitate. Mix well before and during 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 for a record of standards and samples 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 towels.
6. Add 200 µL of Human IL-1β 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 for 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.).

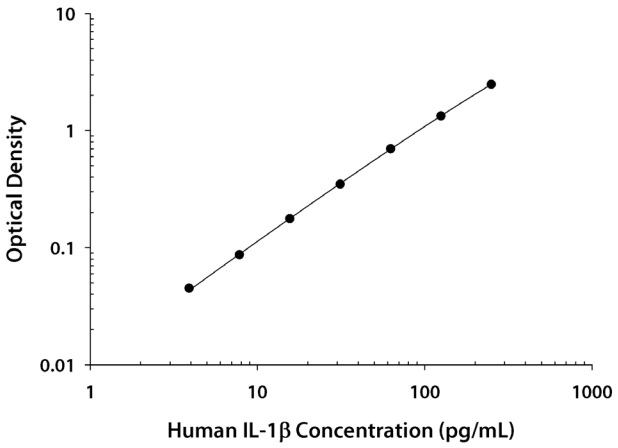
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

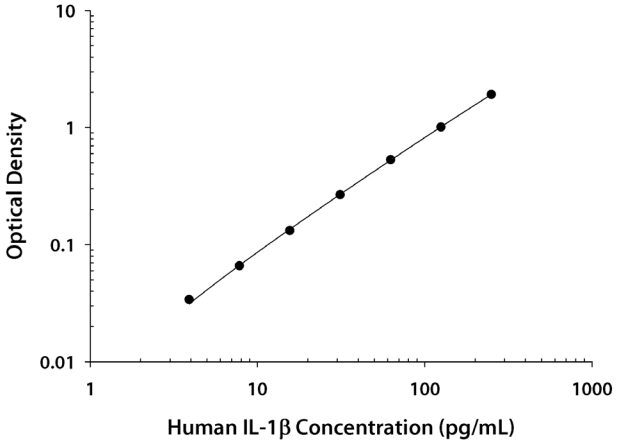
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.027	0.025	—
3.9	0.070 0.071	0.070	0.045
7.8	0.109 0.115	0.112	0.087
15.6	0.197 0.206	0.202	0.177
31.3	0.373 0.374	0.374	0.349
62.5	0.715 0.733	0.724	0.699
125	1.349 1.366	1.358	1.333
250	2.495 2.517	2.506	2.481

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.026	0.024	—
3.9	0.057 0.058	0.058	0.034
7.8	0.088 0.091	0.090	0.066
15.6	0.156 0.157	0.156	0.132
31.3	0.290 0.292	0.291	0.267
62.5	0.549 0.563	0.556	0.532
125	1.034 1.036	1.035	1.011
250	1.933 1.942	1.938	1.914

PRECISION

Intra-assay Precision (Precision within an assay)
Four 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	4	1	2	3
n	20	20	20	20	20	20	20
Mean (pg/mL)	12.6	30.4	69.2	192	30.8	120	194
Standard deviation	0.3	1.0	1.6	6.5	2.2	4.1	7.9
CV (%)	2.4	3.3	2.3	3.4	7.1	3.4	4.1

SERUM/PLASMA ASSAY

	Intra-Assay Precision				Inter-Assay Precision		
Sample	1	2	3	4	1	2	3
n	20	20	20	20	20	20	20
Mean (pg/mL)	18.9	30.2	122	181	29.8	118	193
Standard deviation	1.6	1.0	5.4	5.0	2.5	5.0	8.0
CV (%)	8.5	3.3	4.4	2.8	8.4	4.2	4.1

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media	97	80-111%
Serum	95	87-110%
EDTA plasma	86	81-100%
Heparin plasma	82	76-100%
Citrate plasma	93	83-110%

SENSITIVITY

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

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

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human IL-1β were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=3)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	101	99	99	99	101
	Range (%)	———	98-99	95-101	95-102	96-109
1:4	Average % of Expected	103	97	99	100	99
	Range (%)	103-104	94-100	93-102	96-102	87-104
1:8	Average % of Expected	106	95	100	94	91
	Range (%)	———	93-98	95-106	88-99	87-99
1:16	Average % of Expected	108	89	91	91	87
	Range (%)	107-109	87-91	86-96	84-96	81-93

CALIBRATION

This immunoassay is calibrated against highly purified recombinant human IL-1β. The non-WHO reference material for IL-1β 86/552 was evaluated in this kit. The dose response curve of the reference material parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine kit to approximate NIBSC (86/552) units, use the equation below.

NIBSC (86/552) approximate value (U/mL) = 0.098 x Quantikine Human IL-1β value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Forty serum and plasma samples from apparently healthy volunteers were evaluated for the presence of IL-1β in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest IL-1β standard, 3.9 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with the agents listed in the table below. Aliquots of the cell culture supernate were removed on days 1, 3, and 5 and assayed for levels of human IL-1β.

Stimulant	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
10 µg/mL PHA	2185	2004	2383
10 µg/mL PHA+10 ng/mL rhIL-2	1938	1973	2839
50 ng/mL PMA	1767	1027	1159
50 ng/mL LPS	4158	2145	1308

SPECIFICITY

This assay recognizes natural and recombinant human IL-1β. This assay also recognizes recombinant rhesus macaque IL-1β.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range rhIL-1β control were assayed for interference. No significant cross-reactivity or interference was observed with the following.

Recombinant human:

IL-1α
IL-1F7
IL-1ra
IL-1 RAcP
IL-1 RAPL1
IL-1 RAPL2
IL-1 Rrp2
IL-18
IL-18 Rα
IL-18 Rβ
IL-33
IL-36α
IL-36β
IL-36γ
IL-36ra
IL-38
SIGIRR
ST2

Recombinant mouse:

IL-1α
IL-1β
IL-33
SIGIRR
ST2

Recombinant rat:

IL-1α
IL-1ra

Recombinant porcine:

IL-1α
IL-1β
IL-1ra

Recombinant equine:

IL-1β
IL-1ra

Other recombinants:

cotton rat IL-1α
canine IL-1β
guinea pig IL-1β

Recombinant human Pro-IL-1β cross-reacts approximately 6.1% in this assay.

Recombinant rat IL-1β cross-reacts approximaely 0.2% in this assay.

Recombinant cotton rat IL-1β cross-reacts approximately 0.4% in this assay.

Recombinant human IL-1 RI and IL-1 RII do not cross-react but do interfere at concentrations > 10,000 pg/mL.

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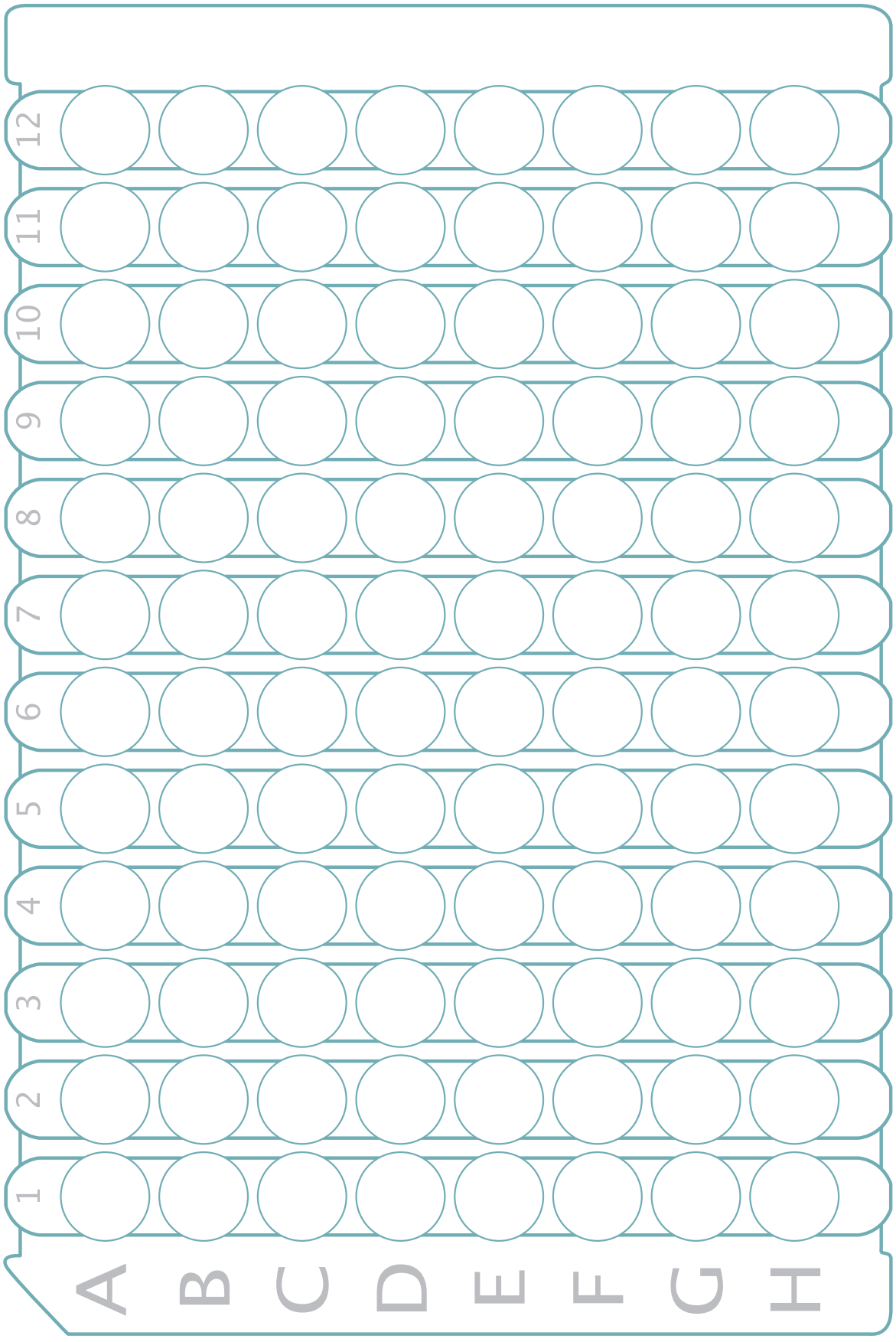
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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