Quantikine® ELISA

Human Pro-IL-1β/IL-1F2 Immunoassay

Catalog Number DLBP00

For the quantitative determination of human Pro-Interleukin 1 beta (Pro-IL-1β) 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

Pro-IL-1β, also known as IL-1b precursor, is a 31 kDa, 269 amino acid (aa) protein precursor of IL-1β (1). It is synthesized in response to most microbes or microbial products as well as other pro-inflammatory stimuli. There is a characteristic dissociation of transcription from production of active protein, with independent regulation of transcription, translation and conversion of the precursor to active protein (1). Pro-IL-1β has no IL-1 bioactivity, and it remains largely in the cytosol prior to secretion from the cell (1, 2). It appears that transport out of the cell is coupled to cleavage into mature, active IL-1β, such that most extracellular IL-1β is in the mature form.

The cleavage of Pro-IL-1β to IL-1β is catalyzed primarily by IL-1β converting enzyme (ICE) (also known as caspase-1), which cleaves between Asp116-Ala117, resulting in a 153 aa, 17.5 kDa, mature IL-1β (residues 117 to 249) (2). Some Pro-IL-1β is also found extracellularly (3), where it is subject to non-specific cleavage at residues close to position 117 by proteases such as trypsin and elastase (1). The mature forms produced by each protease can vary somewhat in size, but are equally active. Extracellular Pro-IL-1β binds with high affinity to soluble IL-1 receptor type II, where it is protected from proteolysis (1).

The Quantikine Human Pro-IL-1β/IL-1F2 is a sandwich enzyme-linked immunoassay. The capture antibody coated to the microplate is specific for the pro-form of the molecule (first 116 residues), and the detection antibody is specific for mature IL-1β. Thus, the assay does not detect either the pro-form alone or the mature form alone; it is specific instead for the intact Pro-IL-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Pro-IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Pro-IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, a rabbit polyclonal antibody specific for human IL-1β is added to the wells. Following a wash to remove any unbound antibody, anti-rabbit IgG-HRP Conjugate is added to the wells. Following a wash to remove any unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of Pro-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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Pro-IL-1β Microplate	890770	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Pro-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 Pro-IL-1β Standard	890772	2 vials of recombinant human Pro-IL-1β in a buffered protein base with preservative; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human Pro-IL-1β Conjugate	890771	11 mL of goat anti-rabbit IgG conjugated to horseradish peroxidase with preservative.	May be stored for up to 1 month at 2-8 °C.*
Human Pro-IL-1β Antiserum	890776	11 mL of a rabbit polyclonal antibody specific for human Pro-IL-1β with preservative.	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5R	895190	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-6	895177	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human Pro-IL-1β Controls (optional; R&D Systems, Catalog # QC145).

PRECAUTIONS

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

Some components in this kit contain ProClin® 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

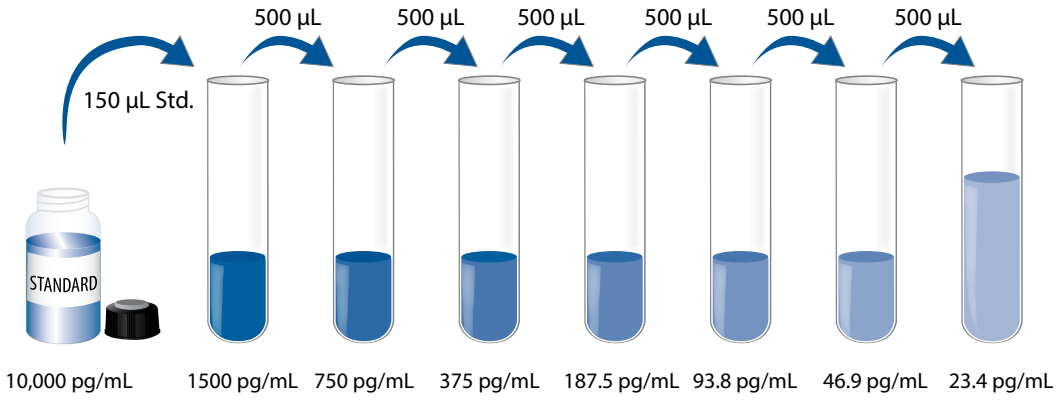
Bring all reagents to room temperature before use.

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.

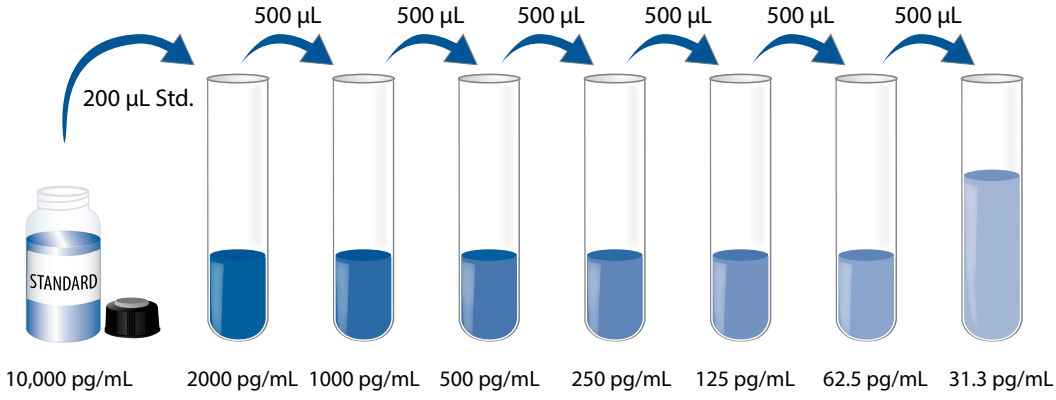
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Pro-IL-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Pro-IL-1β Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. **Note:** *The diluted standard must be used within 60 minutes of preparation.*

For Cell Culture Supernate Samples: Pipette 850 µL of Calibrator Diluent RD5R into the 1500 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1500 pg/mL standard serves as the high standard. Calibrator Diluent RD5R serves as the zero standard (0 pg/mL).



For Serum/Plasma Samples: Pipette 800 µL of Calibrator Diluent RD6-6 into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-6 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 Assay Diluent RD1S to each well.
4. Add 200 µL of Standard, control, or sample per well. Cover with an adhesive strip. Incubate for 1.5 hours at room temperature. A plate layout is provided to record 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 100 µL of Human Pro-IL-1β Antiserum to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Human Pro-IL-1β Conjugate to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
11. 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. 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.).

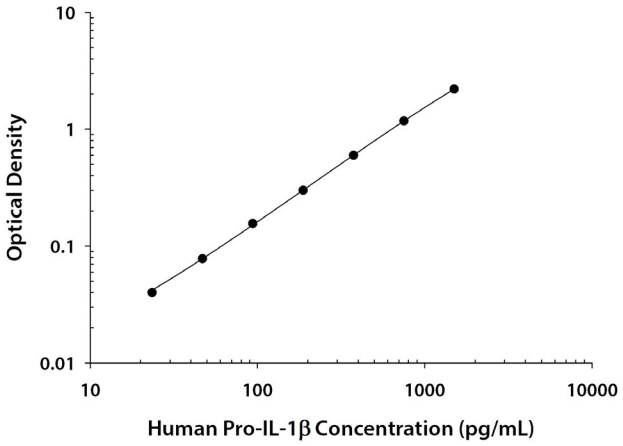
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

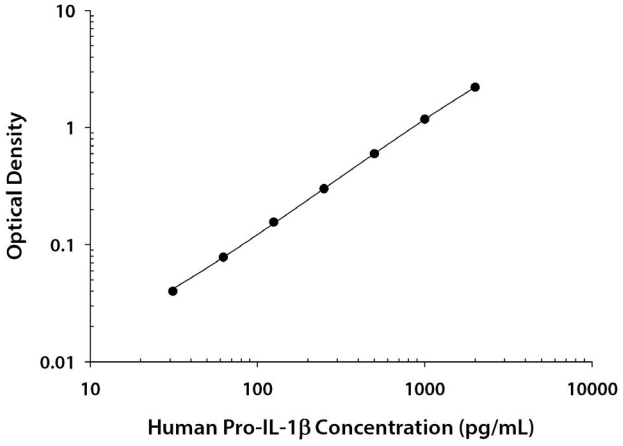
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.021	0.021	—
23.4	0.060 0.062	0.061	0.040
46.9	0.095 0.103	0.099	0.078
93.8	0.183 0.172	0.178	0.157
187.5	0.318 0.324	0.321	0.300
375	0.630 0.608	0.619	0.598
750	1.209 1.182	1.196	1.175
1500	2.207 2.246	2.226	2.205

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.025	0.026	—
31.3	0.066 0.064	0.065	0.039
62.5	0.118 0.115	0.116	0.090
125	0.204 0.202	0.203	0.177
250	0.328 0.346	0.337	0.311
500	0.670 0.687	0.678	0.652
1000	1.264 1.226	1.245	1.219
2000	2.304 2.216	2.260	2.234

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 forty 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	40	40	40
Mean (pg/mL)	85.5	240	432	85.1	248	457
Standard deviation	4.7	11.5	20.8	6.2	14.0	28.3
CV (%)	5.5	4.8	4.8	7.3	5.6	6.2

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	126	374	670	121	342	626
Standard deviation	6.4	15.7	34.5	8.8	17.9	34.9
CV (%)	5.1	4.2	5.1	7.3	5.2	5.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	108	99-115%
Serum (n=5)	102	97-108%
EDTA plasma (n=5)	103	95-108%
Heparin plasma (n=5)	101	92-109%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human Pro-IL-1β in various matrices and diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	100	101	102	103
	Range (%)	92-103	99-105	97-107	98-110
1:4	Average % of Expected	98	103	103	106
	Range (%)	90-103	97-105	98-107	102-110
1:8	Average % of Expected	98	104	100	105
	Range (%)	93-104	100-111	95-107	95-112
1:16	Average % of Expected	94	98	94	101
	Range (%)	90-103	86-110	88-99	94-109

SENSITIVITY

Eleven assays were evaluated using Calibrator Diluent RD6-6 and the minimum detectable dose (MDD) of human Pro-IL-1β ranged from 1.6-8.9 pg/mL with a mean MDD of 5.2 pg/mL. Eleven assays were also evaluated using Calibrator Diluent RD5R and the MDD ranged from 1.2-7.2 pg/mL with a mean MDD of 3.3 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Pro-IL-1β.

SAMPLE VALUES

Serum/Plasma - Sixty serum and thirty plasma samples from apparently healthy volunteers were evaluated for the presence of Human Pro-IL-1β in this assay. All samples measured less than the lowest standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

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, 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA and 10 µg/mL recombinant human IL-2. Aliquots of the cell culture supernates were removed and assayed for levels of human Pro-IL-1β.

Condition	Day 0 (pg/mL)	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	———	218	26.3
Stimulated w/PHA	ND	102	70.0
Stimulated w/PHA + rh IL-2	———	———	318

ND=Non-detectable

Human dendritic cells were cultured in RPMI supplemented with 5% fetal calf serum, 50 ng/mL of recombinant human GM-CSF and 50 ng/mL of recombinant human IL-4. An aliquot of the cell culture supernate was removed, assayed for human Pro-IL-1β, and measured 73.4 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Pro-IL-1β.

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

Recombinant human:

IL-1α
IL-1β
IL-1ra
IL-1 RI
IL-1 RII
IL-2
IL-2 Rα
IL-3
IL-3 Rα
IL-4
IL-4 R
IL-5
IL-5 Rα
IL-5 Rβ
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
IL-18
IL-18 (pro)
ANG
AR
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
GM-CSF
sgp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
KGF
LAP (TGF-β1)
LIF
MCP-1
M-CSF
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β2
TGF-β3
TGF-β RII
TNF-α
TNF-β
TNF RI
TNF RII
VEGF

Recombinant mouse:

IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
GM-CSF
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Natural proteins:

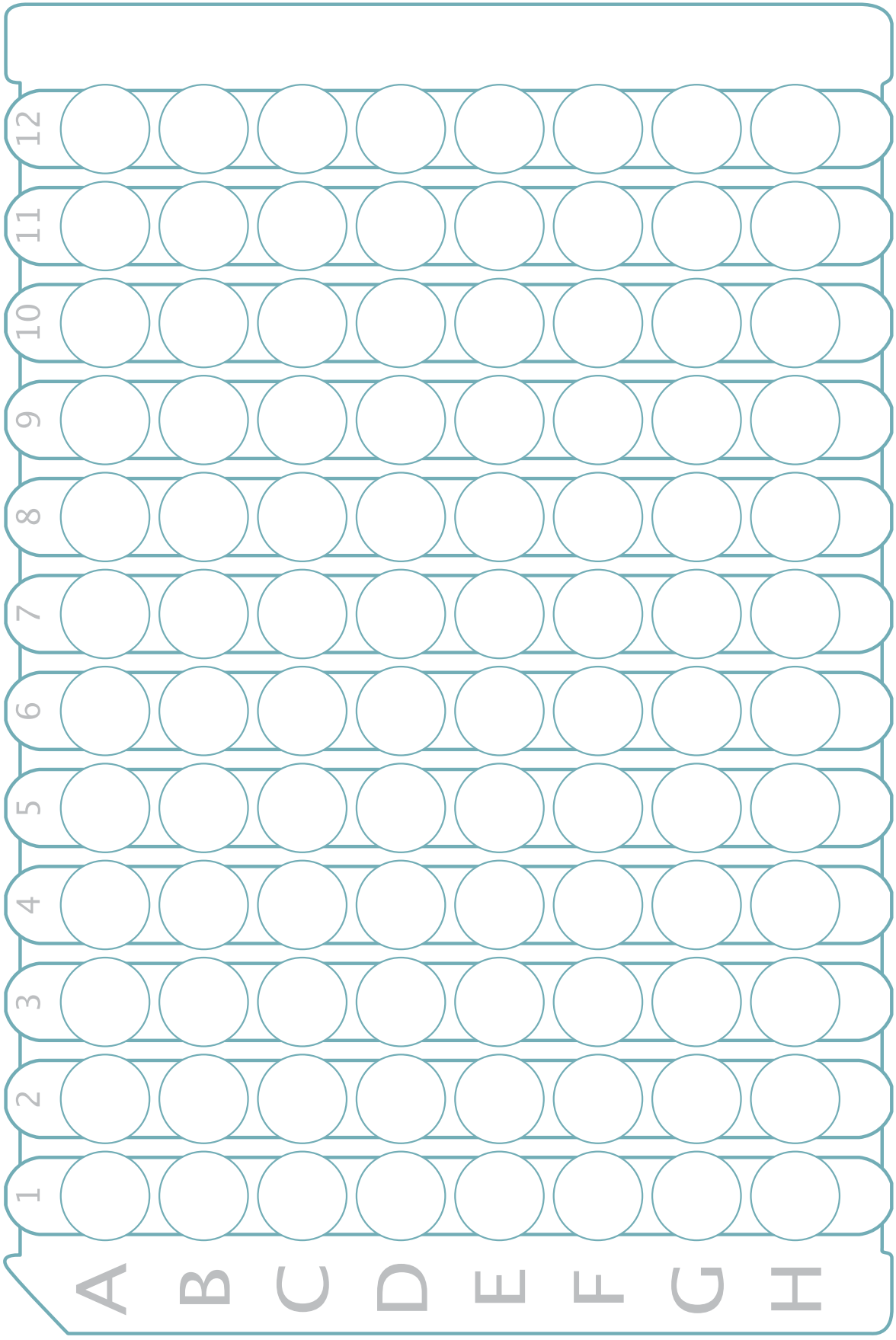
bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1

REFERENCES

1. Dinarello, C.A. (1998) in *The Cytokine Handbook*, 3rd edition, A. Thomson, ed., Academic Press, pp. 35-72.
2. Estrov, Z. and M. Talpaz (1996) Cytokines Molec. Therapy **2**:1.
3. Beuscher, H.U. *et al.* (1990) J. Immunol. **144**:2179.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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