Quantikine® ELISA

Human CXCL8/IL-8 Immunoassay

Catalog Number D8000C
S8000C
PD8000C

For the quantitative determination of human Interleukin 8 (IL-8) 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-8 (IL-8), also known as IL-8, GCP-1, and NAP-1, is a heparin-binding 8-9 kDa member of the alpha, or CXC family of chemokines. There are at least 15 human CXC family members that all adopt a three β-sheet/one α-helix structure. Most CXC chemokines show an N-terminal Glu-Leu-Arg (ELR) tripeptide motif (1, 2). IL-8 circulates as a monomer, homodimer, and heterodimer with CXCL4/PF4. The monomer is considered the most bioactive, while the heterodimer can potentiate PF4 activity (3-6). IL-8 oligomerization is modulated by its interactions with matrix and cell surface glycosaminoglycans (GAGs) (7, 8). Mature human IL-8 shares 65-69% aa identiity with canine, feline, and porcine IL-8 (9, 10). There is no IL-8 gene counterpart in rodent.

Multiple isoforms of IL-8 are generated through both alternative splicing and differential proteolytic cleavage. In humans, alternative splicing generates an isoform with an eleven aa substitution at the C-terminus (11). Proteolytic processing results in N-terminal truncation of IL-8 and is likely a cell-specific event. For example, fibroblasts and endothelial cells generate the 1-77 form by cleaving IL-8 following Glu21, while monocytes and lymphocytes generate the 6-77 form by cleaving following Leu25. These truncated forms generally show increased bioactivity, particularly through the CXCR1 receptor (12-14). IL-8 can also undergo citrullination on Arg27 of the precursor, a modification that increases its half-life and ability to induce leukocytosis (15, 16). A wide variety of cells secrete IL-8 including monocytes and neutrophils (17), fibroblasts and keratinocytes (18), mast cells (19), visceral smooth muscle cells (20), dendritic cells (21), type II great alveolar cells (22), and endothelial cells (23).

IL-8 bioactivity is mediated through two G-protein-coupled receptors, termed CXCR1/IL-8 RA and CXCR2/IL-8 RB (24). CXCR1 is 45-50 kDa in size and is used almost exclusively by IL-8. CXCR2 is 35-40 kDa in size and is used by nearly all CXC chemokines (25, 26). Both CXCR1 and CXCR2 constitutively associate into functional homodimers. They can also heterodimerize, but these complexes dissociate following IL-8 binding (27). CXCR2 responds to low concentrations of IL-8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, in contrast, responds to high concentrations of IL-8 and is associated with respiratory burst and phospholipase D2 activation (26). Thus, CXCR2 ligation induces leukocyte adhesion to activated vascular endothelium and migration to sites of inflammation, while CXCR1 ligation primes neutrophil antimicrobial activity (28). IL-8 can also form a complex with Serpin A1/alpha-1 Antitrypsin, and this prevents IL-8 interaction with CXCR1 (29).

In addition to its proinflammatory effects, IL-8 is involved in angiogenesis and the pathogenesis of atherosclerosis and cancer (30-33). It induces VEGF expression in vascular endothelial cells and functions as an autocrine factor for EC growth and angiogenesis (34, 35). It is upregulated in atherosclerotic lesions and is elevated in the serum and cerebrospinal fluid following myocardial infarction (36, 37). In cancer, IL-8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (32, 38-40).

The Quantikine Human CXCL8/IL-8 Immunoassay is a 3.5 hour solid phase ELISA designed to measure human IL-8 in cell culture supernates, serum, and plasma. It is based on antibodies raised against the 72 aa variant of human IL-8 derived from *E. coli*. It is calibrated with the same recombinant factor. This immunoassay accurately quantitates recombinant human IL-8. Measurement of natural human IL-8 or the 77 aa variant of human IL-8 gave results parallel to the standard curves obtained using the *E. coli*-expressed Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-8.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-8 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-8 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 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.
- 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 # D8000C	CATALOG # S8000C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-8 Microplate	890462	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-8.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-8 Conjugate	890465	1 vial	6 vials	11 mL/vial of a polyclonal antibody specific for human IL-8 conjugated to horseradish peroxidase with red dye and preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-8 Standard	890466	1 vial	6 vials	10 ng/vial of recombinant human IL-8 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-85	895877	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Used diluted 1:5 in this assay. For cell culture supernate samples.*	
Calibrator Diluent RD6Z	895466	1 vial	6 vials	21 mL/vial of diluted 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.

D8000C contains sufficient materials to run an ELISA on one 96 well plate.
S8000C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PD8000C). 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.

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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human IL-8 Controls (optional; available from R&D Systems).

PRECAUTIONS

IL-8 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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, 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.

SAMPLE PREPARATION

Cell culture supernate samples may require up to a 100-fold dilution prior to assay. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5).*

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of IL-8 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

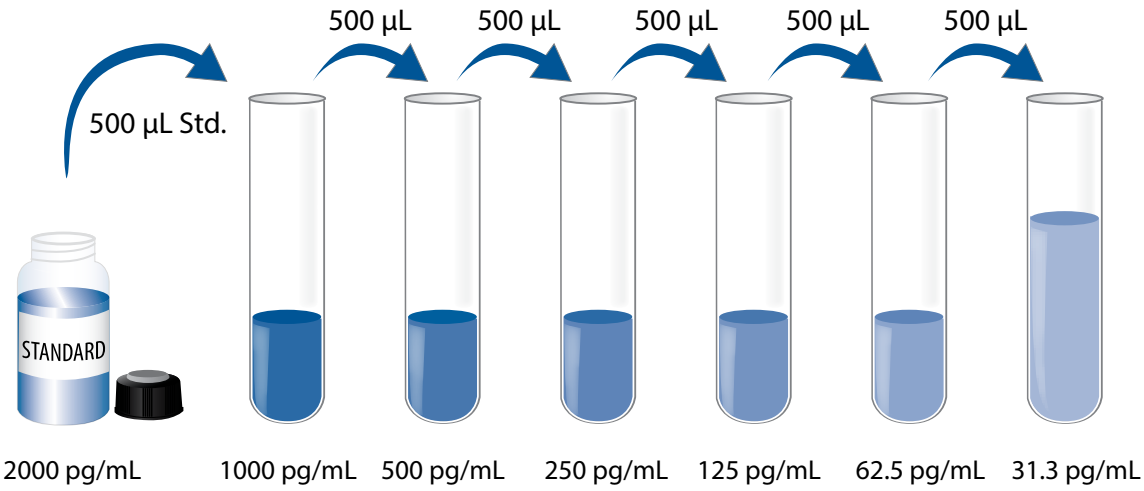
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - **For cell culture supernate samples.** Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human IL-8 Standard - Reconstitute the Human IL-8 Standard with 5.0 mL of Calibrator Diluent RD5P (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6Z (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 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 RD5P (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6Z (*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 standard serves as the high standard (2000 pg/mL). 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: *High concentrations of IL-8 are found in saliva. It is recommended that a face mask and gloves be used to protect the assay from contamination.*

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 100 µL of Assay Diluent RD1-85 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Securely cover with a plate sealer and incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 IL-8 Conjugate to all wells. Securely cover with a plate sealer and incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 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 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.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

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

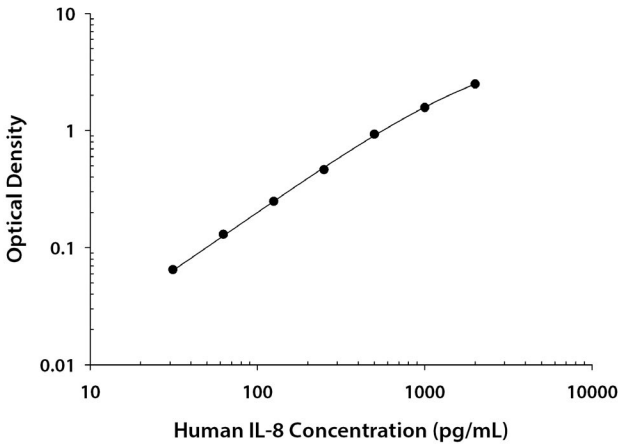
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-8 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

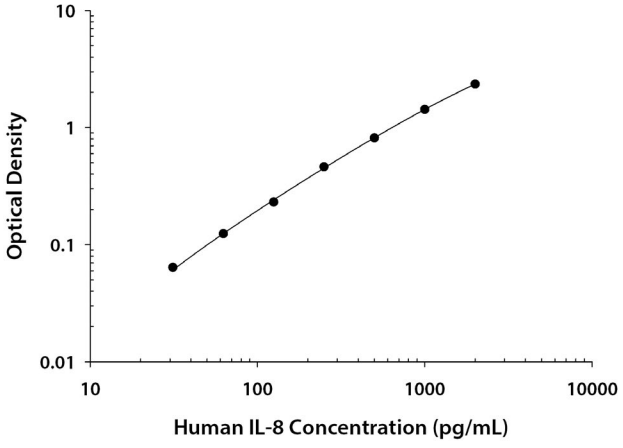
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.099 0.101	0.100	—
31.3	0.164 0.166	0.165	0.065
62.5	0.227 0.232	0.230	0.130
125	0.347 0.350	0.349	0.249
250	0.559 0.570	0.564	0.464
500	1.025 1.036	1.030	0.930
1000	1.662 1.681	1.671	1.571
2000	2.588 2.606	2.597	2.497

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.048 0.050	0.049	—
31.3	0.111 0.114	0.113	0.064
62.5	0.170 0.175	0.173	0.124
125	0.275 0.285	0.280	0.231
250	0.509 0.512	0.511	0.462
500	0.836 0.893	0.865	0.816
1000	1.451 1.503	1.477	1.428
2000	2.387 2.416	2.402	2.353

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)	115	386	802	132	410	817
Standard deviation	5.3	17.0	37.9	10.7	28.0	42.4
CV (%)	4.6	4.4	4.7	8.1	6.8	5.2

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)	168	526	1093	196	581	1160
Standard deviation	9.4	28.6	70.7	14.5	56.6	70.2
CV (%)	5.6	5.4	6.5	7.4	9.7	6.1

RECOVERY

The recovery of human IL-8 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	98	85-114%
Serum (n=5)	98	88-106%
EDTA plasma (n=5)	103	97-111%
Heparin plasma (n=5)	102	92-107%
Citrate plasma (n=5)	105	95-114%

SENSITIVITY

Fifty-three assays were evaluated and the minimum detectable dose (MDD) of human IL-8 ranged from 1.5-7.5 pg/mL. The mean MDD was 3.5 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 the linearity of the assay, samples were spiked with high concentrations of human IL-8 in various matrices and diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	96	95	93	93	93
	Range (%)	93-99	93-98	89-97	89-97	87-96
1:4	Average % of Expected	100	94	94	95	92
	Range (%)	99-101	91-103	89-98	89-98	86-100
1:8	Average % of Expected	104	94	94	94	91
	Range (%)	93-110	90-110	89-105	85-106	83-104
1:16	Average % of Expected	106	93	95	94	91
	Range (%)	95-116	84-101	89-103	86-102	83-101

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed 72 amino acid (monocyte) form of recombinant human IL-8 produced at R&D Systems.

The NIBSC/WHO 1st International Standard 89/520 was evaluated in this kit. The dose response curve of this standard parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human IL-8 kit to approximate NIBSC International Units, use the equation below.

NIBSC (89/520) approximate value (IU/mL) = 0.001 x Quantikine Human IL-8 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Thirty-four samples from apparently healthy volunteers were evaluated for the presence of IL-8 in this assay. All samples measured less than the lowest IL-8 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, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of natural human IL-8.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	27,000	33,000
Stimulated	73,000	102,000

SPECIFICITY

This assay recognizes natural and recombinant human IL-8.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IL-8 control were assayed for 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
GM-CSF
GROα
GROβ
GROγ
gp130
HB-EGF
HGF
I-309
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-1 RI
IL-1 RII
IL-2
IL-2 Rα
IL-3
IL-3 Rα
IL-4 R
IL-5
IL-5 Rβ
IL-6
IL-6 R
IL-7
IL-9
IL-10
IL-11
IL-12
IL-13
IP-10
KGF
LAP (TGF-β1)
LIF
M-CSF
MCP-1
MCP-2
MCP-3
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PF-4
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β3
TGF-β RII
TNF-α
TNF-β
TNF RI
TNF RII
VEGF

Recombinant mouse:

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

Recombinant amphibian:

TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β2

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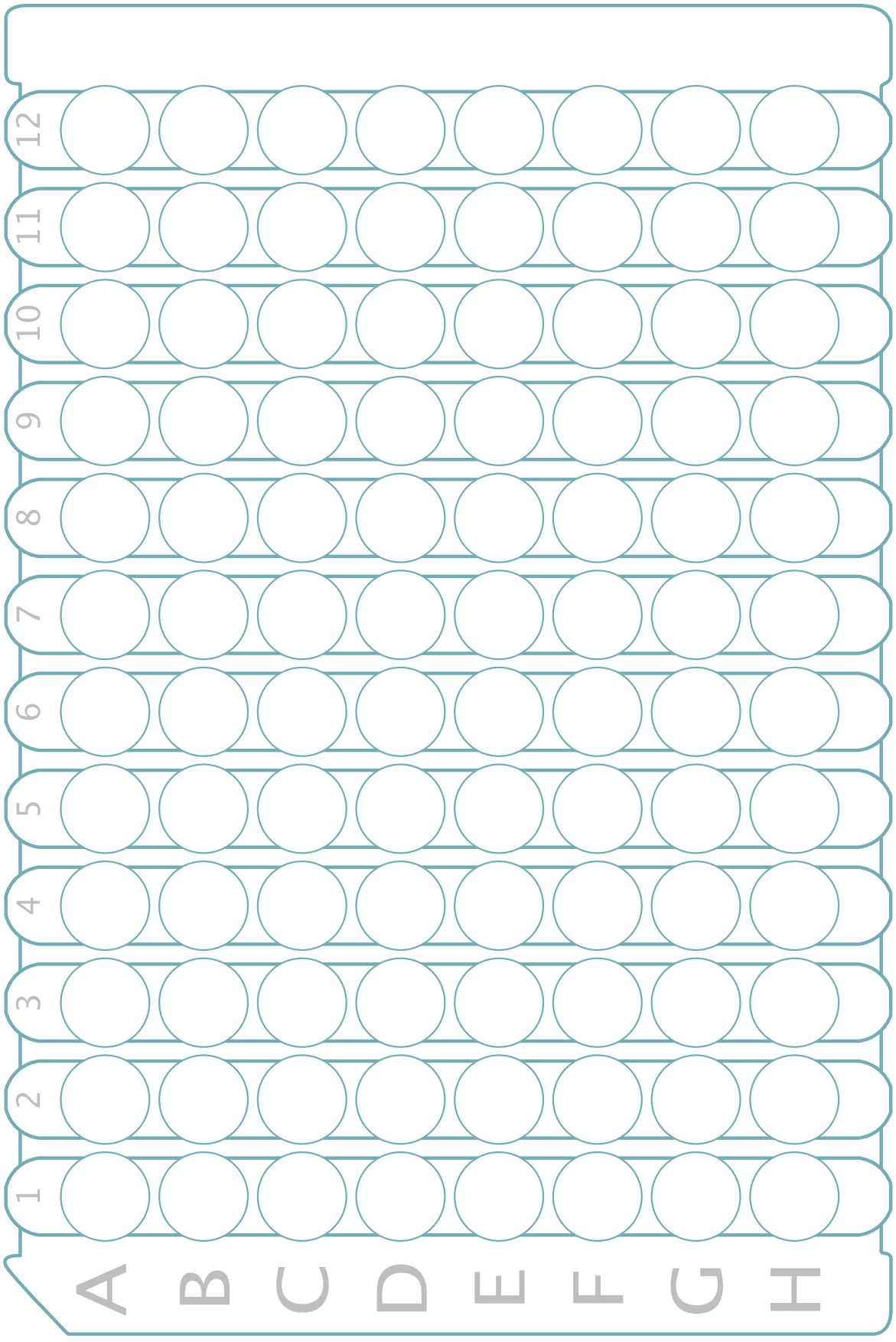
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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