Quantikine™ ELISA

Human IL-6 Immunoassay

Catalog Number D6050B
S6050B
PD6050B

For the quantitative determination of human Interleukin 6 (IL-6) concentrations in cell culture supernates, serum, plasma, and urine.

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

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	3
TECHNICAL HINTS	3
MATERIALS PROVIDED & STORAGE CONDITIONS	4
PHARMPAK CONTENTS	5
OTHER SUPPLIES REQUIRED	5
PRECAUTIONS	6
SAMPLE COLLECTION & STORAGE	6
SAMPLE PREPARATION	6
REAGENT PREPARATION	7
ASSAY PROCEDURE	8
CALCULATION OF RESULTS	9
TYPICAL DATA	9
PRECISION	10
RECOVERY	10
SENSITIVITY	10
LINEARITY	11
CALIBRATION	11
SAMPLE VALUES	12
SPECIFICITY	12
REFERENCES	13
PLATE LAYOUT	14

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INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic, α-helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature human IL-6 is 183 amino acids (aa) in length and shares 39% aa sequence identity with mouse and rat IL-6 (6). Alternative splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties (7-10). Cells known to express IL-6 include CD8+ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells (2, 11-33). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2). Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (34-38).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R alpha) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization (39). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (40). Soluble forms of IL-6 Rα are generated by both alternative splicing and proteolytic cleavage (5). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 Rα elicit responses from gp130-expressing cells that lack cell surface IL-6 Rα (5). Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα but not from other cytokines that use gp130 as a co-receptor (5, 41).

IL-6, along with TNF-α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-5). When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis (2, 5). IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity (1). It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis (1, 2). IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise (2). In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells (42).

INTRODUCTION *CONTINUED*

The Quantikine™ Human IL-6 Immunoassay is a 4.5 hour solid phase immunoassay designed to measure human IL-6 in cell culture supernates, serum, plasma, and urine. It contains recombinant human IL-6 and antibodies raised against the recombinant protein. Natural human IL-6 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine kit standards, indicating that this kit can be used to determine relative levels of natural human IL-6.

It has been observed in our laboratories that the measurement of IL-6 is insensitive to the addition of the recombinant form of the IL-6 soluble receptor. Therefore it is probable that experimental sample measurements reflect the total amount of IL-6 present, i.e., the total amount of free IL-6 plus the amount of IL-6 initially bound to soluble receptors, if any are present in the samples. High levels of high-affinity autoantibodies to IL-6 in the serum of some blood donors have been reported (36, 37). Such autoantibodies have the potential to interfere with the measurement of IL-6 by ELISA immunoassays.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-6 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-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

For research use only. Not for use in diagnostic procedures.

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, further dilute the samples with 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 # D6050B	CATALOG # S6050B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 Microplate	899519	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-6.	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-6 Standard	899521	2 vials	12 vials	Recombinant human IL-6 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IL-6 Conjugate	899520	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IL-6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
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 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

D6050B contains sufficient materials to run an ELISA on one 96 well plate.
S6050B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD6050B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IL-6 Microplate	899519	50 plates
Human IL-6 Standard*	899521	25 vials
Human IL-6 Conjugate	899520	50 vials
Assay Diluent RD1W	895117	50 vials
Calibrator Diluent RD5-3	895436	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- 500 mL graduated cylinder
- Squirt bottle, manifold dispenser, or automated microplate washer
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human IL-6 Controls (optional; R&D Systems, Catalog # QC304)

PRECAUTIONS

IL-6 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 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 SDS 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 this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernates may require dilution due to high endogenous levels. Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

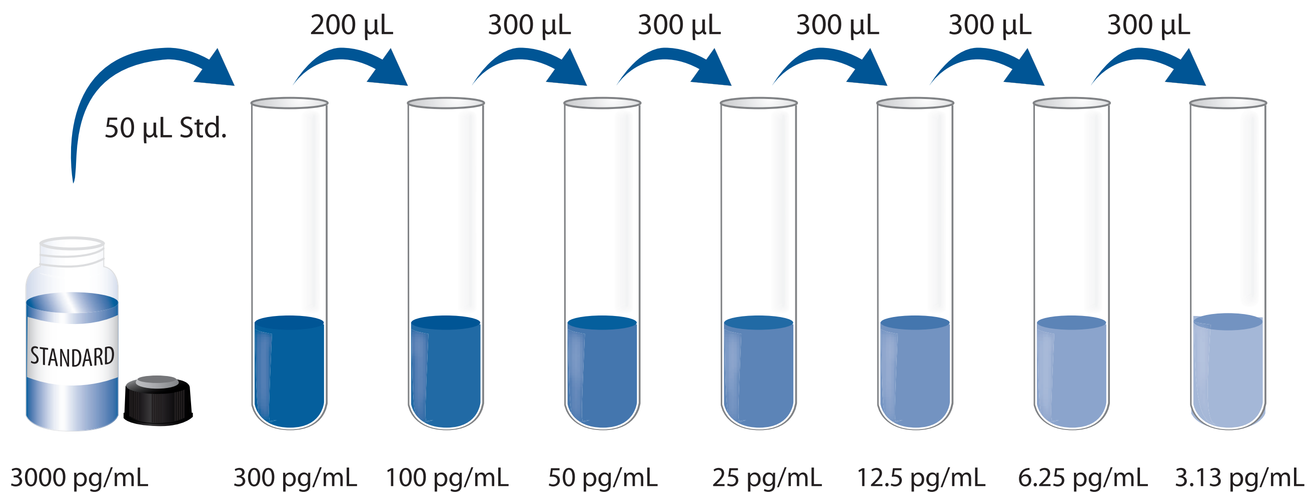
Note: *High concentrations of IL-6 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents from contamination.*

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 480 mL of 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-6 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-6 Standard with deionized or distilled water. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-3 into the 300 pg/mL tube. Pipette 400 µL of Calibrator Diluent RD5-3 into the 100 pg/mL tube and 300 µL into remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 300 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 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, controls, and samples be assayed in duplicate.

Note: *High concentrations of IL-6 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents 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 RD1W to each well.
4. Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 200 µL of Human IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 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.

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

CALCULATION OF RESULTS

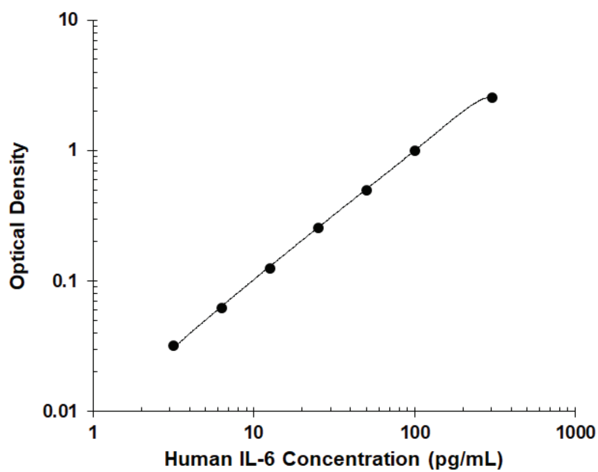
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-6 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.005	0.005	—
3.13	0.032 0.032	0.032	0.027
6.25	0.063 0.063	0.063	0.058
12.5	0.124 0.125	0.125	0.120
25	0.255 0.263	0.259	0.254
50	0.504 0.504	0.504	0.499
100	0.987 1.010	0.999	0.994
300	2.569 2.575	2.572	2.567

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	38.8	94.7	205	31.8	97.4	191
Standard deviation	0.687	2.02	4.69	1.35	3.65	8.25
CV (%)	1.8	2.1	2.3	4.2	3.8	4.3

RECOVERY

The recovery of human IL-6 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	82-122
Serum (n=4)	96	89-103
EDTA plasma (n=4)	97	90-102
Heparin plasma (n=4)	96	90-99
Urine (n=4)	97	89-101

SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.120-0.626 pg/mL. The mean MDD was 0.368 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 the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-6 in various matrices were diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernate (n=3)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	97	104	103	105	100
	Range (%)	81-109	94-112	95-111	98-111	98-105
1:4	Average % of Expected	100	106	105	106	101
	Range (%)	88-116	97-115	95-115	98-115	98-104
1:8	Average % of Expected	107	108	107	110	100
	Range (%)	95-121	99-118	99-115	102-120	98-103
1:16	Average % of Expected	116	109	108	109	101
	Range (%)	99-125	103-119	101-116	99-117	98-105

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IL-6 produced at R&D Systems®.

The NIBSC/WHO Interleukin-6 International Standard 89/548 (IL-6, Human rDNA derived) was evaluated in this kit. The dose response curve of the International Standard (89/548) parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human IL-6 kit to approximate NIBSC 89/548 units, use the equation below.

NIBSC (89/548) approximate value (IU/mL)=0.1006 x Quantikine Human IL-6 value (pg/mL)

Based on data generated from April 2023.

SAMPLE VALUES

Serum/Plasma- Samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=30)	6.90	40	ND-13.9
EDTA plasma (n=30)	6.82	40	ND-13.4
Heparin plasma (n=30)	6.89	40	ND-12.9

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. PBMCs were left untreated or treated with 10 ug/mL PHA for 5 days before collecting conditioned media. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-6.

Condition	Day 5 (pg/mL)
Unstimulated	24.0
Stimulated	10,986

Urine - Ten samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IL-6 standard, 3.13 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-6.

The factors listed below were prepared at 3 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 3 ng/mL in a mid-range recombinant human IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

gp130
IL-6 Rα/gp130
IL-6 sR
IL-6 R

Other recombinants:

mouse IL-6
rat IL-6
porcine IL-6

Recombinant cyno macaque IL-6 cross-reacts at approximately 79% and interferes at levels > 50 pg/mL.

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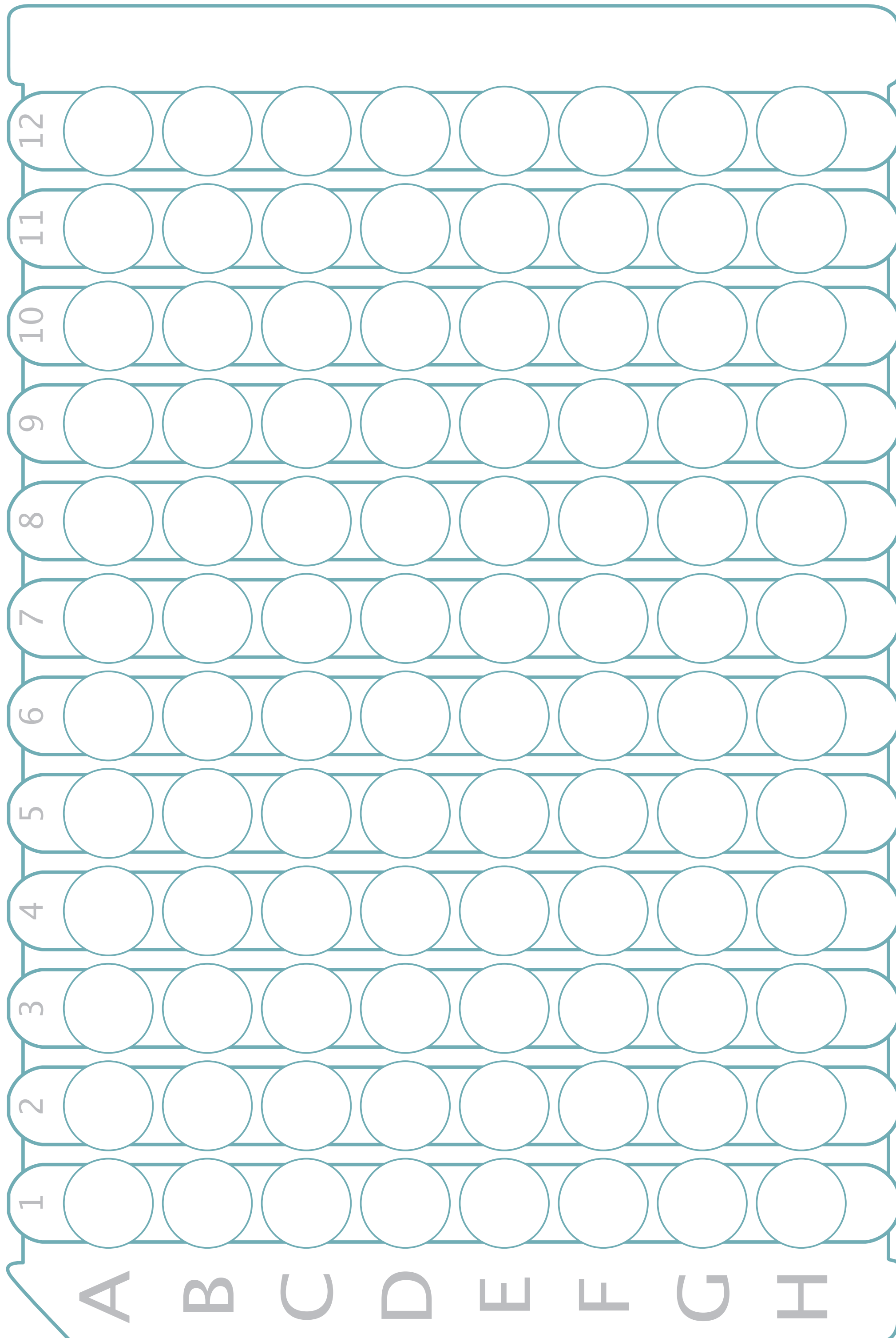
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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