Quantikine® ELISA

Human IL-12/IL-23 p40 Immunoassay

Catalog Number DP400
SP400
PDP400

For the quantitative determination of human Interleukin 12 p40 (IL-12 p40) 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.

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INTRODUCTION

IL-12, also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF), is a pleiotropic cytokine produced primarily by antigen-presenting cells (monocytes/macrophages, dendritic cells and B lymphocytes). IL-12 has multiple effects on T lymphocytes and natural killer (NK) cells, including the ability to stimulate cytotoxicity, proliferation, cytokine production and Th1 subset development (1, 2).

IL-12 is a disulfide-linked, 70 kDa (p70) heterodimeric glycoprotein composed of a 40 kDa (p40) subunit and a 35 kDa (p35) subunit. The p40 and p35 subunits by themselves do not have IL-12 activity, but the homodimer of p40 has been shown to bind the IL-12 receptor and is an IL-12 antagonist (3, 4).

The genes for human p40 and p35, found on chromosomes 5 and 3, respectively, are independently regulated (1, 5). The expression of p35 mRNA has been found to be nearly ubiquitous, however, p35 subunits have not been detected in culture supernates of cells expressing only p35 or both p35 and p40 mRNAs (1). In cells expressing both p35 and p40 mRNAs, p40 mRNA is expressed to a higher level and free p40 subunits not associated with p35 subunits are secreted together with heterodimeric IL-12 p70 (6). Most of the free p40 subunits secreted by the various human cell lines examined have been found to exist as monomers (1). In the culture supernates of various activated human monocytes where free p40 is present in vast excess over p70, the levels of p70 measured by bioassays are consistent with those measured using a p70-specific immunoassay, suggesting that p40 monomers are not efficient IL-12 antagonists (1, 7). Currently, the physiological role of free p40 subunits is not clear.

The Quantikine® Human IL-12/IL-23 p40 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure IL-12/IL-23 p40 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human IL-12/IL-23 p40 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-12/IL-23 p40. Results obtained using natural human IL-12/IL-23 p40 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-12/IL-23 p40.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-12/IL-23 p40 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12/IL-23 p40 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-12 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-12/IL-23 p40 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 # DP400	CATALOG # SP400	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-12/IL-23 p40 Microplate	890512	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-12/IL-23 p40.	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-12/IL-23 p40 Conjugate	890513	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-12 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-12/IL-23 p40 Standard	890514	1 vial	6 vials	Recombinant human IL-12/IL-23 p40 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	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. *For cell culture supernate samples. Use diluted 1:5 in this assay.*	
Calibrator Diluent RD6-13	895491	1 vial	6 vials	21 mL/vial of a buffered protein base 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.

DP400 contains sufficient materials to run an ELISA on one 96 well plate.
SP400 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDP400). 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.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- **Polypropylene** test tubes for dilution of standards.
- Human IL-12/IL-23 p40 Controls (optional; R&D Systems®, Catalog # QC22).

PRECAUTIONS

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.

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.

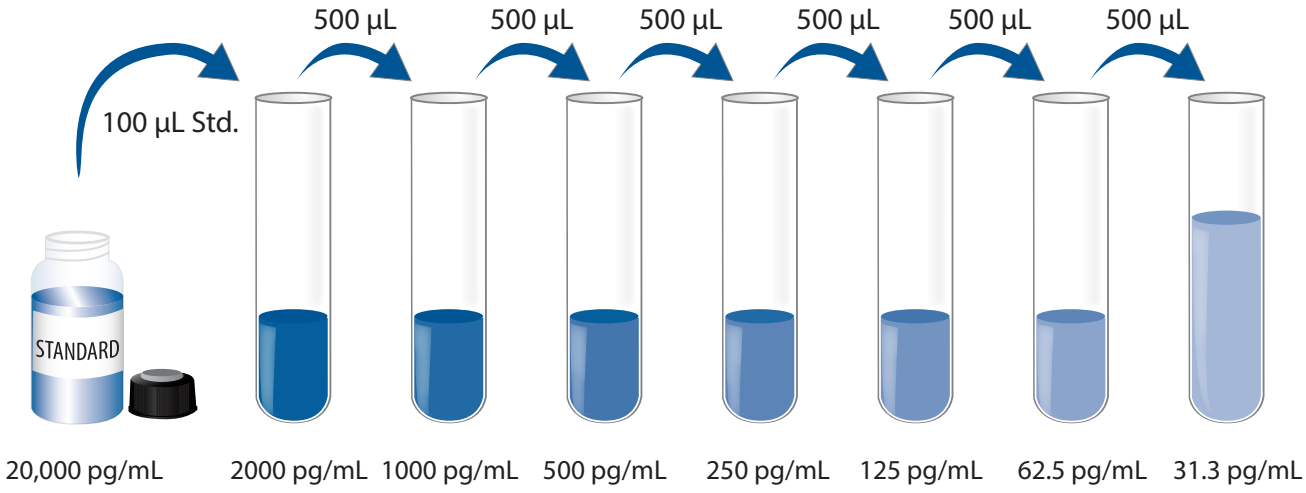
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - 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-12/IL-23 p40 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-12/IL-23 p40 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6-13 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent 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. 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 100 µL of Assay Diluent RD1W to each well.
4. Add 100 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 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 200 µL of Human IL-12/IL-23 p40 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 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 at 450 nm. If wavelength correction is available, set at 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.).

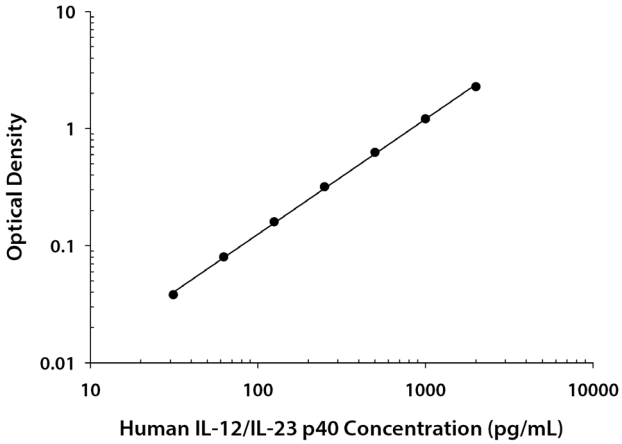
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-12/IL-23 p40 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

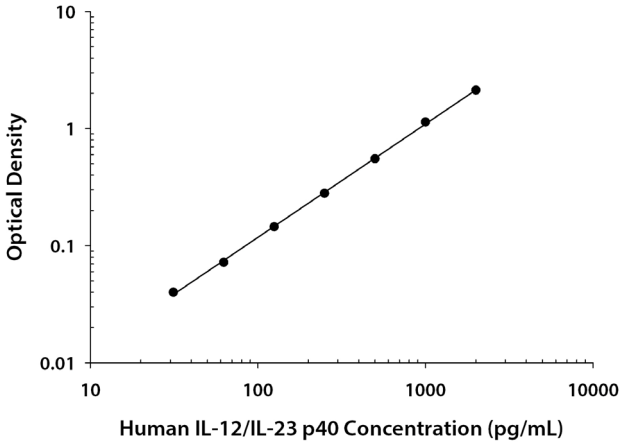
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.044 0.048	0.046	—
31.3	0.085 0.084	0.084	0.038
62.5	0.127 0.126	0.126	0.080
125	0.202 0.209	0.206	0.160
250	0.362 0.367	0.364	0.318
500	0.674 0.671	0.672	0.626
1000	1.256 1.247	1.252	1.206
2000	2.359 2.278	2.318	2.272

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.050 0.050	0.050	—
31.3	0.091 0.089	0.090	0.040
62.5	0.123 0.121	0.122	0.072
125	0.193 0.197	0.195	0.145
250	0.326 0.333	0.330	0.280
500	0.593 0.612	0.602	0.552
1000	1.167 1.210	1.188	1.138
2000	2.175 2.178	2.176	2.126

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)	119	290	871	127	315	891
Standard deviation	5.1	11.4	35.5	11.8	19.8	60.6
CV (%)	4.3	3.9	4.1	9.3	6.3	6.8

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)	151	380	1138	160	399	1145
Standard deviation	9.9	27.2	62.3	11.0	22.3	63.0
CV (%)	6.6	7.2	5.5	6.9	5.6	5.5

RECOVERY

The recovery of human IL-12/IL-23 p40 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=5)	96	85-105%
Serum (n=5)	98	86-112%
EDTA plasma (n=5)	101	92-113%
Heparin plasma (n=5)	96	89-106%
Citrate plasma (n=5)	100	85-112%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-12/IL-23 p40 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)	Citrate plasma (n=5)
1:2	Average % of Expected	101	98	95	99	96
	Range (%)	98-104	92-103	91-101	96-104	90-103
1:4	Average % of Expected	103	96	95	97	96
	Range (%)	98-107	87-98	88-98	90-103	90-102
1:8	Average % of Expected	101	95	93	97	93
	Range (%)	91-114	86-99	84-97	87-101	86-102
1:16	Average % of Expected	97	97	92	96	94
	Range (%)	89-103	91-105	81-103	90-105	86-103

SENSITIVITY

The minimum detectable dose (MDD) of human IL-12/IL-23 p40 is typically less than 15 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-12/IL-23 p40 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-12/IL-23 p40 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=94)	91	ND-390
EDTA plasma (n=35)	99	ND-415
Heparin plasma (n=35)	87	ND-339
Citrate plasma (n=35)	90	ND-354

ND=Non-detectable

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. The 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-12/IL-23 p40.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	121	520
Stimulated	420	552

Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated for 1.5 days with the agents listed in the table below. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-12/IL-23 p40.

Stimulant	Day 1.5 (pg/mL)
1 µg/mL LPS	910
100 ng/mL rhIFN-γ	528
1 µg/mL LPS + 100 ng/mL rhIFN-γ	15,333
0.0075% SAC	3066
0.0075% SAC + 100 ng/mL rhIFN-γ	11,469

SPECIFICITY

This assay recognizes natural and recombinant human IL-12/IL-23 p40.

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 recombinant human IL-12/IL-23 p40 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF
gp130
IL-6
IL-6 Rα
IL-12
IL-12 Rβ1
IL-12 Rβ2
IL-23 R
IL-27
IL-35
TCCR

Recombinant mouse:

gp130
IL-12
IL-12/IL-23 p40
IL-12/IL-23 p40 Homodimer
IL-12/IL-23 p40 Monomer
IL-12 p70
IL-12 Rβ
IL-12 Rβ2
IL-23
IL-23 p19
IL-23 R
IL-27
IL-27 p28
TCCR

Recombinant human IL-12 p70 cross-reacts approximately 0.05% in this assay.

Recombinant human IL-23 cross-reacts approximately 0.9% in this assay.

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5. Sieburth, D. *et al.* (1992) Genomics **14**:59.
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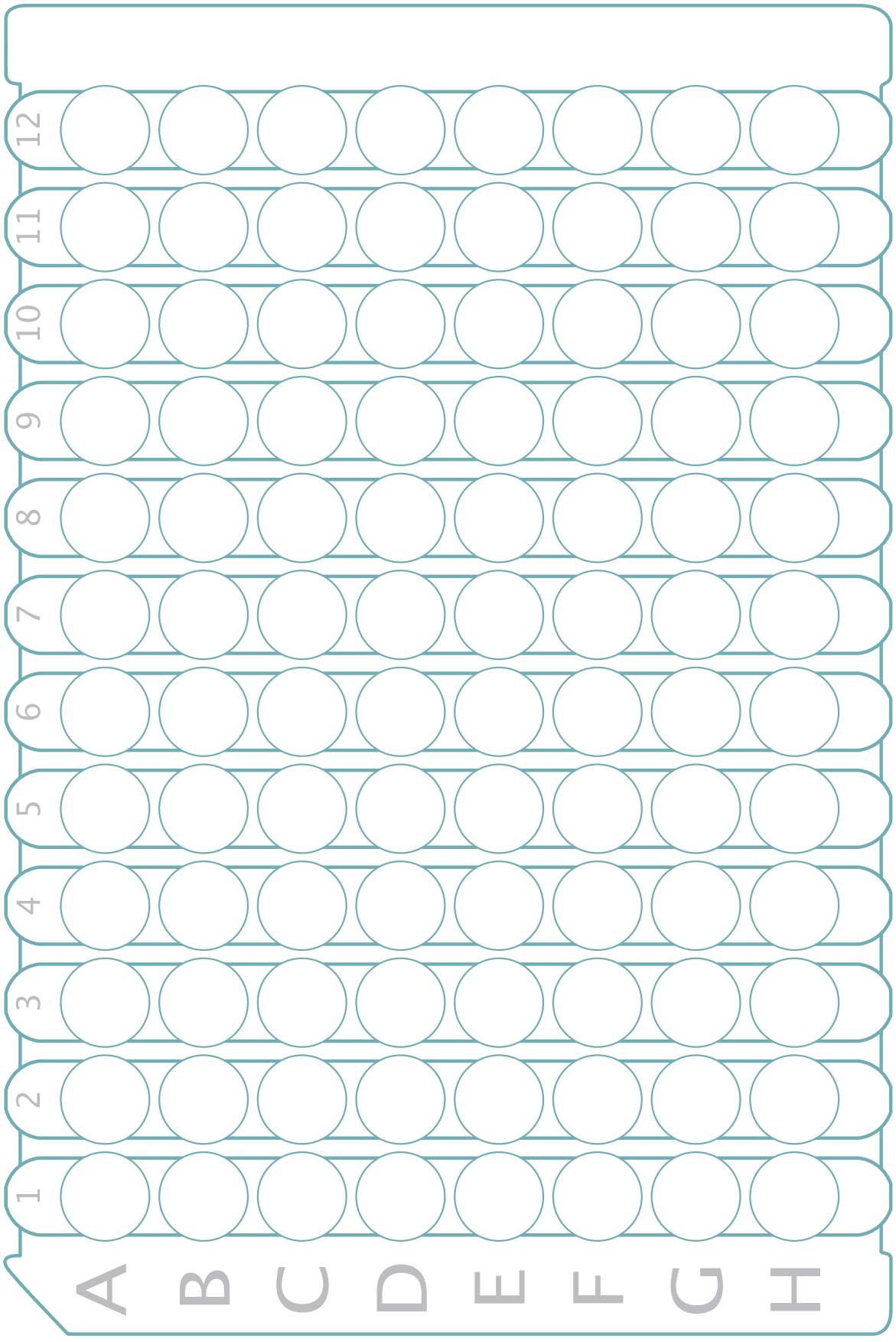
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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