Quantikine® ELISA

Human IL-28B/IFN-λ3 Immunoassay

Catalog Number D28B00

For the quantitative determination of human Interleukin 28B (IL-28B) concentrations in cell culture supernates, cell lysates, serum, EDTA 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	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	5
SAMPLE PREPARATION	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS	8
TYPICAL DATA	8
PRECISION	9
RECOVERY	9
LINEARITY	10
SENSITIVITY	10
CALIBRATION	10
SAMPLE VALUES	11
SPECIFICITY	12
REFERENCES	13
PLATE LAYOUT	14

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INTRODUCTION

IL-28B, also known as IFN-λ3, belongs to the type III interferon (also called IFN-λ) family of class II cytokine receptor ligands. This family of cytokines, which also includes IL-28A and IL-29, is structurally related to the IL-10 family and type I interferon family (1, 2). Much like type I interferons, the type III interferons are immune regulatory proteins with significant anti-viral, anti-proliferative, and anti-tumor activity (3-6). Type III interferons are detectable in a wide variety of tissues and can be induced in antigen presenting cells upon infection with virus, treatment with lipopolysaccharide or Poly I:C, or direct stimulation with IFN-γ (1, 7-9).

Human IL-28B cDNA encodes a 200 amino acid (aa) protein with a 25 aa signal peptide and 175 aa mature protein that lacks N-glycosylation sites. Mature human IL-28B shares 94% and 69% aa identity with human IL-28A and IL-29, respectively (2). Mature human IL-28B shares 64% aa sequence identity with mouse IL-28B. IL-28B mediates its biological activities through a heterodimeric receptor complex composed of the IL-10 receptor beta (IL-10 Rβ) and the IL-28 receptor alpha (IL-28 Rα). This receptor is expressed on epithelial cells, dendritic cells, T cells, and plasmacytoid dendritic cells (10). IL-28B first binds to IL-28 Rα causing a conformational change in the receptor-ligand complex that facilitates the recruitment of IL-10 Rβ (11). The activated receptor induces JAK1 and TYK2 tyrosine kinase activation, STAT phosphorylation, and formation of the IFN-stimulated regulatory factor 3 (ISGF-3) transcription complex. Upon translocation to the nucleus, ISGF3 binds to IFN-stimulated response elements to induce expression of anti-viral-associated genes (11).

Type III interferons are known for their potent anti-viral activity, which includes the upregulation of MHC class I expression on antigen presenting cells as well as increasing expression of Toll-like Receptors (TLRs) and factors that enhance TLR signaling (12). IL-28B is thought to be a biomarker for infection; it is often increased in human serum during viral infection and in the sputum and lung fluids of asthmatics (10, 13, 14). Polymorphisms in IL-28B correlate with its ability to respond to viral infection. IL-28B polymorphisms are also thought to correspond with treatment outcomes for anti-viral therapies against chronic hepatitis C infection (15-17). Other immunoregulatory activities of IL-28B include modulating the Th1 and Th2 responses during infection and enhancing peripheral B cell proliferation (12, 18). IL-28B enhances adaptive immunity by reducing regulatory T cell populations and increasing $CD8^+$ T cell proliferation (19).

The Quantikine® Human IL-28B/IFN-λ3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure IL-28B levels in cell culture supernates, cell lysates, serum, EDTA plasma, and urine. It contains CHO cell-expressed recombinant human IL-28B and antibodies raised against the recombinant protein. Results obtained for naturally occurring human IL-28B 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 human IL-28B.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-28B has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-28B present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-28B 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-28B 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, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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 IL-28B Microplate	894897	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-28B.	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-28B Standard	894899	2 vials of recombinant human IL-28B 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-28B Conjugate	894898	21 mL of a monoclonal antibody specific for human IL-28B conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-9	895167	11 mL of a buffered protein solution with preservatives. *May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein base with preservatives.	
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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards and samples.
- Human IL-28B Controls (optional; R&D Systems®, Catalog # QC222).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Lysis Buffer 17 (R&D Systems®, Catalog # 895943).

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 SDS on our website prior to use.

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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.

Cell Lysates - Cells must be lysed prior to assay as directed in the Sample Values section.

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 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: *Heparin and citrate plasma have not been validated for use in this assay.*
Grossly lipemic samples are not suitable for use in this assay.

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

SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate and urine samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 150 μL of sample + 150 μL of Calibrator Diluent RD5-3.

For cell lysate samples, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 25-200 μg/well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

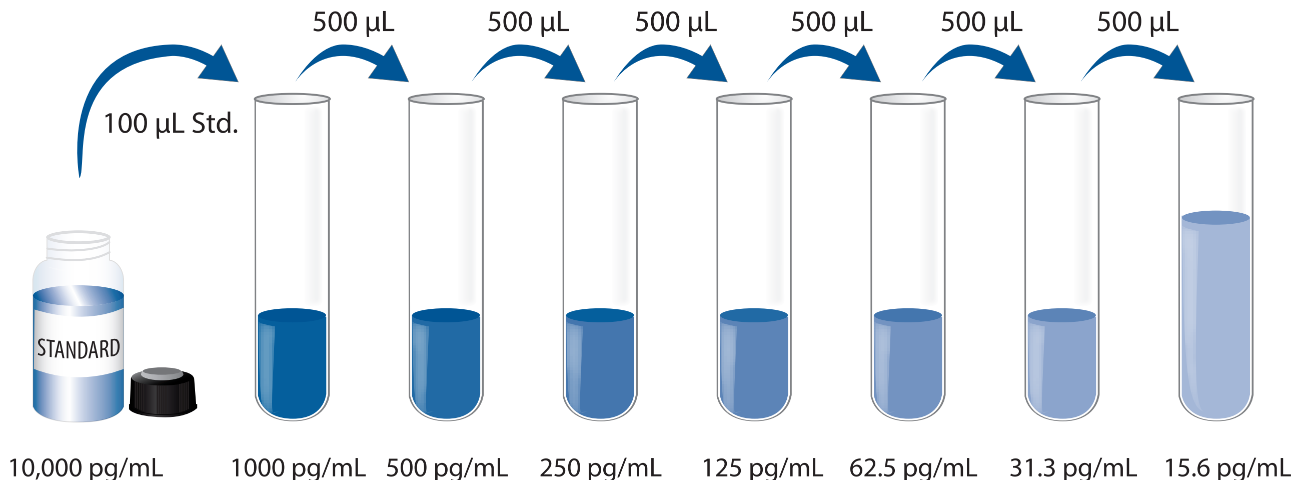
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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-28B Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-28B Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Note: *Do not use rocker.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-3 into the 1000 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 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).



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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.

1. Prepare all reagents, working standards, and samples 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 of Assay Diluent RD1-9 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. 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 the Human IL-28B Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at 2-8 °C without shaking.**
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 **on the benchtop. 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 Sample Preparation section.

CALCULATION OF RESULTS

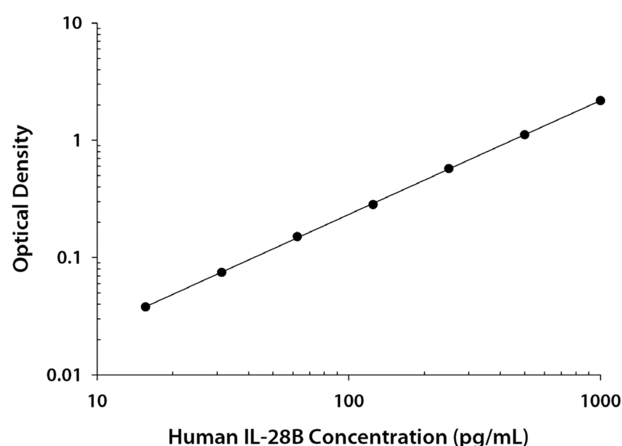
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-28B 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	0.010 0.010	0.010	—
15.6	0.047 0.048	0.048	0.038
31.3	0.084 0.085	0.085	0.075
62.5	0.160 0.162	0.161	0.151
125	0.291 0.295	0.293	0.283
250	0.581 0.587	0.584	0.574
500	1.123 1.125	1.124	1.114
1000	2.188 2.188	2.188	2.178

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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)	90.7	279	560	98.6	282	532
Standard deviation	2.80	10.5	11.9	7.15	19.9	36.1
CV (%)	3.1	3.8	2.1	7.3	7.1	6.8

RECOVERY

The recovery of human IL-28B spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media* (n=4)	113	83-122%
Lysis buffer (n=4)	96	87-103%
Serum (n=4)	111	96-124%
EDTA plasma (n=4)	111	93-124%
Urine* (n=4)	84	75-96%

*Samples were diluted prior to assay as described in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-28B were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Lysis buffer (n=4)	Serum (n=4)	EDTA plasma (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	103	94	96	104
	Range (%)	88-123	99-110	92-96	92-105	97-113
1:4	Average % of Expected	95	101	88	92	105
	Range (%)	82-117	100-105	81-93	86-105	92-118
1:8	Average % of Expected	91	96	83	88	102
	Range (%)	77-113	93-100	80-87	82-102	87-113
1:16	Average % of Expected	88	91	81	90	100
	Range (%)	78-107	85-102	79-82	82-97	92-105

*Samples were diluted prior to assay as described in the Sample Preparation section.

SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human IL-28B ranged from 0.313-1.87 pg/mL. The mean MDD was 1.05 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 highly purified CHO cell-derived recombinant human IL-28B produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Thirty-six serum and EDTA plasma samples, and 12 urine samples from apparently healthy volunteers were evaluated for the presence of human IL-28B in this assay. No medical histories were available for the donors used in this study. No detectable levels were observed.

Cell Culture Supernates - Human peripheral blood leukocytes (PBL) were cultured in RPMI and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate overnight. Cells were then cultured stimulated with 10 µg/mL PHA and 10 ng/mL recombinant human IL-2 and grown until confluent. An aliquot of the cell culture supernate was removed, and assayed for human IL-28B, and measured 94.8 pg/mL.

Cell Culture Supernates/Cell Lysates - A549 human lung carcinoma cells were cultured in Kaighn's F12 media and supplemented with 10% fetal bovine serum and grown to ~90% confluence. Cells were then cultured unstimulated or stimulated with 10 µg/mL poly I:C and delivered via a lipofectamine for 24 hours. For cell lysates, Lysis Buffer 17 was added on ice for 15-30 minutes with gentle agitation and then centrifuged to remove debris. Aliquots of the cell culture supernates and cell lysates were removed and assayed for human IL-28B.

Condition	Cell culture supernates (pg/mL)	Cell lysates (pg/mL)
Unstimulated	ND	ND
Stimulated	1362	360

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-28B.

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-28B control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IFN-α/β R1
IFN-α/β R2
IFN-α2
IFN-γ
IFN-γ R1
IL-10 Rβ
IL-22
IL-28A
IL-28 Rα
IL-29

Recombinant mouse:

IFN-γ R1
IFN-κ
IL-10 Rβ
IL-28A
IL-28B
IL-28 Rα

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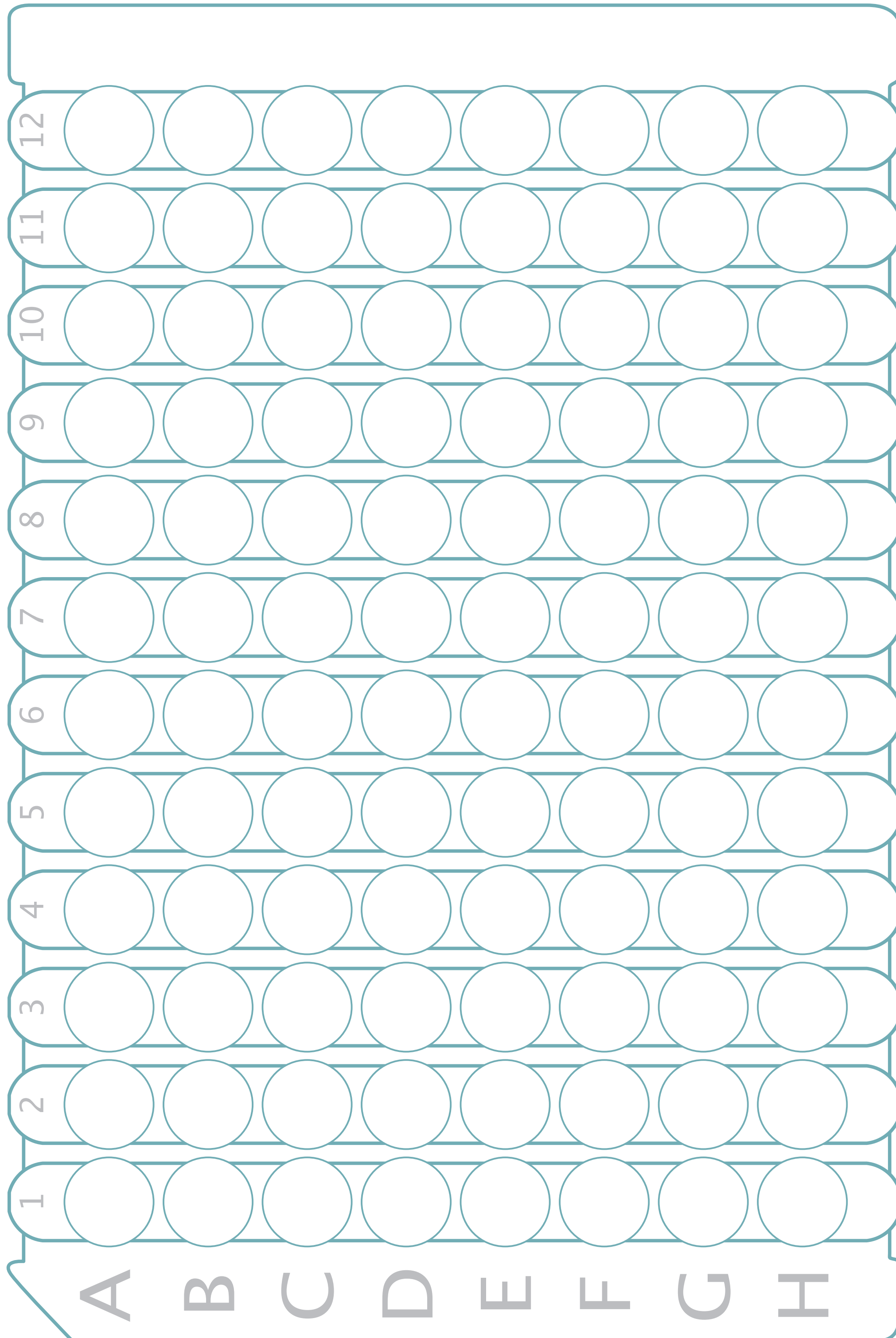
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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