Quantikine® ELISA

Human IL-15 Immunoassay

Catalog Number D1500
S1500
PD1500

For the quantitative determination of human Interleukin 15 (IL-15) 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 15 (IL-15) is a novel cytokine that shares many biological properties with IL-2. It was originally identified in media conditioned by a monkey kidney epithelial cell line (CVI/EBNA) based on its mitogenic activity on the mouse T cell line, CTLL-2 (1). IL-15 was independently discovered as a cytokine produced by a HuT 102 human cutaneous T cell lymphocyte cell line that stimulated T cell proliferation and was designated IL-T (2). Descriptions of the discovery and properties of IL-15 can be found in several reviews (3-5).

Human, simian and mouse IL-15 cDNAs have been isolated and characterized (1, 6, 7). The IL-15 cDNA clones from all three species encode a 162 amino acid (aa) residue precursor protein that contains a 48 aa leader sequence and a 114 aa mature IL-15 subunit. In humans, an alternative splice form has also been identified. This isoform has an identical mature sequence, but a shorter signal sequence. Notably, the shorter prepeptide is not secreted and may represent a functional intracellular molecule (8, 9). In addition, a membrane-bound form of IL-15 has also been reported, which may be an important physiological form of the molecule (10). Human IL-15 shares approximately 97% and 73% aa sequence identity with simian and mouse IL-15, respectively. Both human and simian IL-15 are active on mouse cells.

High-affinity cell surface receptors for IL-15 have been detected on a variety of cells including T cells, B cells, and NK cells as well as on non-lymphoid cells (11, 12). The IL-15 receptor is composed of three molecules; an IL-15 specific α-chain, plus a β-chain and γ-chain that is shared by the receptor system for IL-2. The α-chain is 237 aa in length and binds IL-15 with high affinity. It shows 54% aa sequence identity with mouse IL-15 Rα (13, 14) and exists in multiple alternatively spliced forms (14, 15). Although the IL-15 receptor is composed of 3 chains, IL-15 will signal through a βγ dimer (14) and the α-chain itself may have multiple functions (16).

IL-15 has biological activities similar to IL-2 and has been shown to stimulate the growth of natural killer cells, activated peripheral blood T lymphocytes (1, 11, 12), tumor infiltrating lymphocytes (TILs) (17), and B cells (18). In addition, IL-15 has also been shown to be a chemoattractant for human blood T lymphocytes (19) and to induce both lymphokine-activated killer (LAK) activity in NK cells and the generation of cytolytic effector cells. IL-15 apparently also has effects on cells not involved in immune responses. Skeletal muscle cells express IL-15 and IL-15 R mRNAs and respond to the addition of IL-15 (20).

The Quantikine® Human IL-15 Immunoassay is a 4.25-4.5 hour solid phase ELISA designed to measure human IL-15 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-15 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-15 showed linear 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-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-15 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-15 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-15 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-15 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 # D1500	CATALOG # S1500	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-15 Microplate	890463	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-15.	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-15 Conjugate	890467	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IL-15 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-15 Standard	890468	2 vials	12 vials	Recombinant human IL-15 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-19	895467	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-10	895468	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.

D1500 contains sufficient materials to run an ELISA on one 96 well plate.
S1500 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD1500). 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.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human IL-15 Controls (optional; R&D Systems®, Catalog # QC26).

PRECAUTIONS

Calibrator Diluent RD6-10 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 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 is not recommended for use in this assay.*

REAGENT PREPARATION

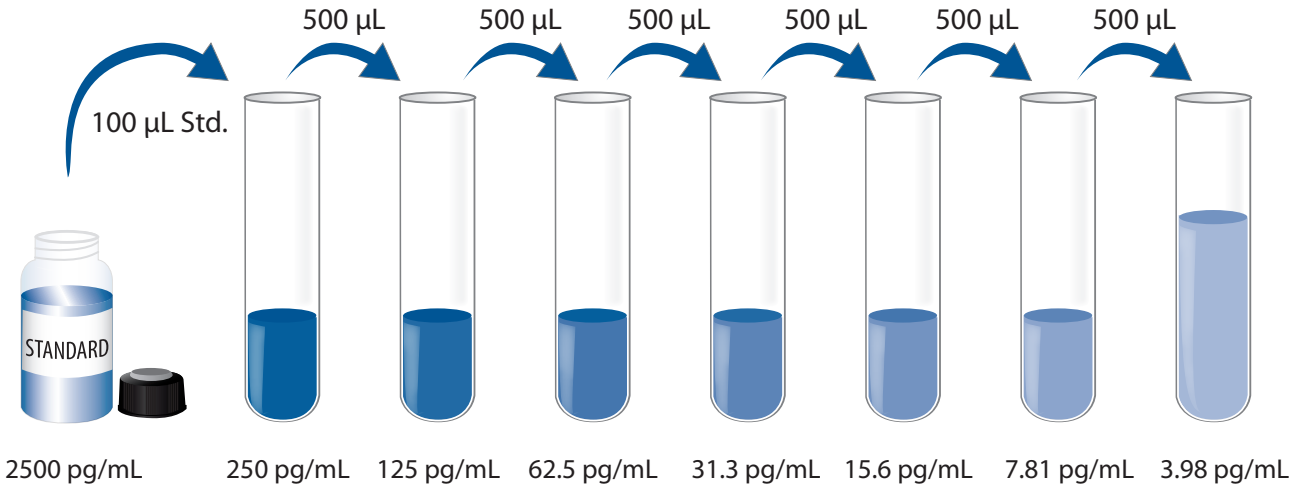
Bring all reagents to room temperature before use.

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 IL-15 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-15 Standard with deionized water. This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Do not continually rotate Standard.

Pipette 900 µL of the Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-10 (*for serum/plasma samples*) into the 250 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 250 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, 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 100 µL of Assay Diluent RD1-19 to each well.
4. Add 50 µL of standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 3 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-15 Conjugate to each well. Cover with a new adhesive strip. **For Cell Culture Supernates Samples:** Incubate for 45 minutes at room temperature. **For Serum/Plasma Samples:** 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 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.

CALCULATION OF RESULTS

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

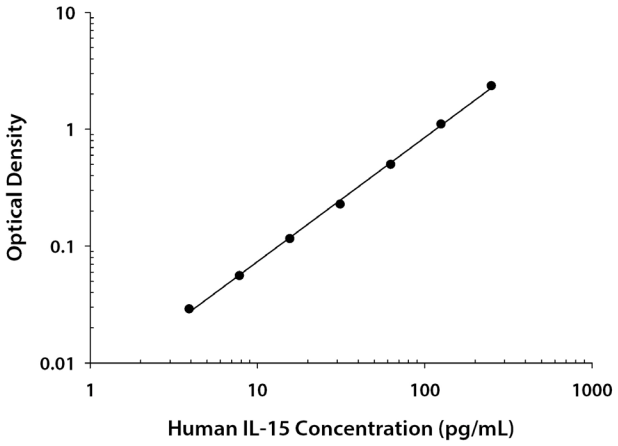
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-15 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

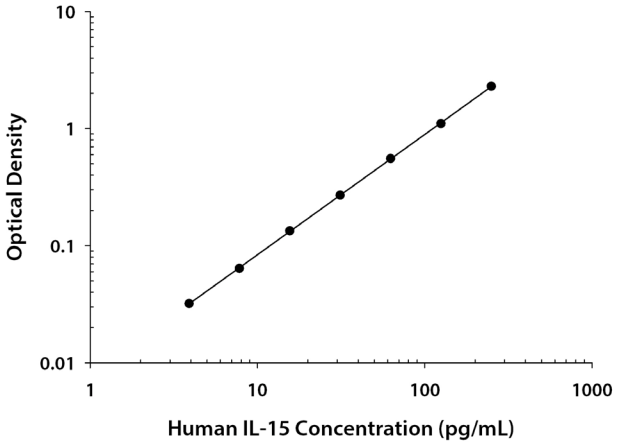
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.036	0.036	—
3.98	0.067 0.063	0.065	0.029
7.81	0.093 0.091	0.092	0.056
15.6	0.154 0.151	0.152	0.116
31.3	0.269 0.260	0.264	0.228
62.5	0.531 0.540	0.536	0.500
125	1.158 1.130	1.144	1.108
250	2.408 2.378	2.393	2.357

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.039 0.037	0.038	—
3.98	0.070 0.071	0.070	0.032
7.81	0.100 0.105	0.102	0.064
15.6	0.174 0.169	0.172	0.134
31.3	0.318 0.299	0.308	0.270
62.5	0.587 0.598	0.592	0.554
125	1.173 1.106	1.140	1.102
250	2.404 2.263	2.334	2.296

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)	7.2	22.9	131	9.2	29.0	154
Standard deviation	0.3	1.3	5.8	0.7	1.6	10.2
CV (%)	4.2	5.7	4.4	7.6	5.5	6.6

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)	7.7	24.7	140	9.9	32.8	184
Standard deviation	0.3	1.3	4.8	0.9	2.7	12.2
CV (%)	3.9	5.3	3.4	9.1	8.2	6.6

RECOVERY

The recovery of human IL-15 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	93-104%
Serum (n=5)	98	92-109%
EDTA plasma (n=5)	99	90-112%
Heparin plasma (n=5)	100	91-107%

LINEARITY

To assess linearity of the assay, samples spiked with high concentrations of human IL-15 were diluted with the appropriate 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	102	100	96	92
	Range (%)	98-107	93-112	88-106	87-99
1:4	Average % of Expected	98	100	97	93
	Range (%)	92-101	94-110	89-112	89-99
1:8	Average % of Expected	96	99	97	95
	Range (%)	93-99	92-115	88-113	89-104
1:16	Average % of Expected	95	99	93	92
	Range (%)	90-101	89-114	84-105	83-107

SENSITIVITY

The minimum detectable dose (MDD) of human IL-15 is typically less than 2 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 *E. coli*-expressed recombinant human IL-15 produced at R&D Systems®.

The NIBSC/WHO Reference Reagent, recombinant human IL-15, preparation 95/554 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-15 kit to approximate NIBSC 95/554 units, use the equation below.

NIBSC (95/554) approximate value (U/mL) = 0.0175 x Quantikine® Human IL-15 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Thirty-seven serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-15 in this assay. All samples measured less than the lowest IL-15 standard, 3.9 pg/mL. No medical histories were available for the donors used in this study.

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 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural human IL-15. All samples measured less than the lowest IL-15 standard, 3.9 pg/mL.

HuT 102 human cutaneous T cell lymphocyte cells were cultured in a 1:1 mixture of DMEM with high glucose and Hamm's F12 supplemented with a 150-fold final dilution of Nutridoma NS (Boehringer Mannheim) and a 500-fold dilution of defined lipid concentrate (Gibco). An aliquot of the cell culture supernate was removed, assayed for human IL-15, and measured 153 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-15.

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-15 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-2 Rβ
IL-2 Rγ
IL-3
IL-3 Rα
IL-4
IL-4 Rα
IL-5
IL-5 Rα
IL-5 Rβ
IL-6
IL-6 R
IL-7
IL-7 R
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13

Recombinant mouse:

GM-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
IL-15
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant human IL-15 Rα (aa 1-172) + recombinant human IL-15 (aa 49-162) complex cross-reacts at approximately 21%.

Recombinant human IL-15 Rα interferes at concentrations > 10 ng/mL.

Recombinant mouse IL-15 Rα interferes at concentrations > 10 ng/mL.

Recombinant mouse IL-15 + recombinant mouse IL-15 Rα complex interferes at concentrations > 10 ng/mL.

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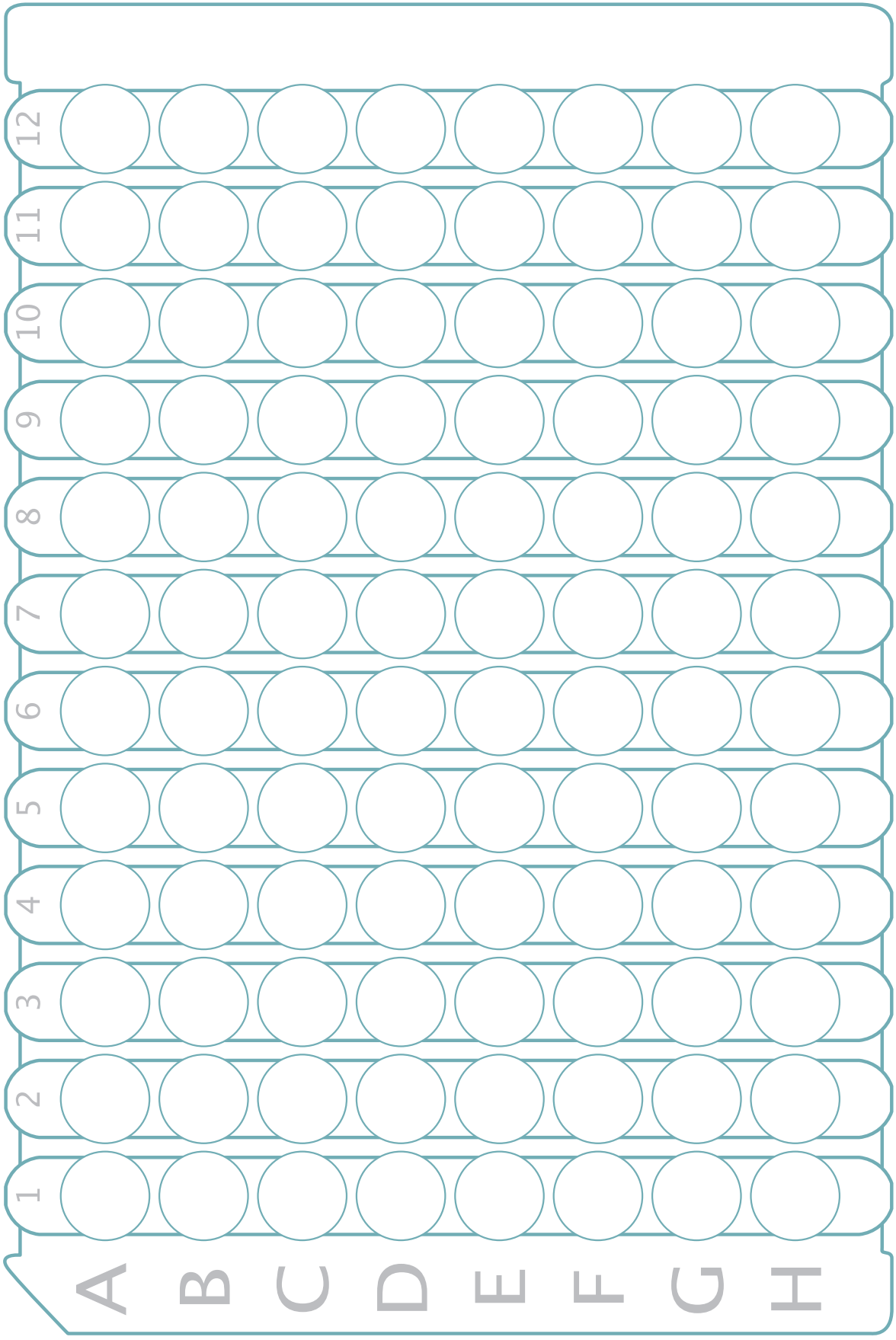
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES