Quantikine® ELISA

Human IL-3 Immunoassay

Catalog Number D3000

For the quantitative determination of human Interleukin 3 (IL-3) 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 3 (IL-3) is a pleiotropic factor that can stimulate the proliferation, differentiation, and survival of pluripotent hematopoietic stem cells as well as various lineage-committed progenitors (1, 2). It was originally purified and characterized based on its ability to induce the production of the enzyme 20α-steroid dehydrogenase in mouse splenocytes (3, 4). Following its purification and cloning, it became apparent that IL-3 had been studied under different names including mast cell growth factor (MCGF), P-cell stimulating factor, hematopoietic cell growth factor (HCGF), burst promoting activity (BPA), multi-colony stimulating factor (multi-CSF), thy-1 inducing factor, and WEHI-3 growth factor. IL-3 is expressed by several hematopoietic and non-hematopoietic cell types including activated human T cells (5, 6), natural killer cells (7), mast cells (8), epithelial cells (9), stromal cells (10), murine keratinocytes (11), neurons (12) and astrocytes (12).

The nucleotide sequence for human IL-3 predicts a mature polypeptide of 133 amino acids (aa) and a 19 aa signal sequence (13). Natural human IL-3 purified from Jurkat cells is a glycosylated protein with observed molecular weights ranging from 14.6 to 30 kDa depending on the amount of glycosylation (14). The gene for IL-3 has been mapped to human chromosome 5 and is closely linked to the genes for IL-4, IL-5, and GM-CSF (15). At the aa level, there is only 29% homology between mouse and human IL-3 (13, 16). Consistent with this lack of homology, the proteins are species-specific in their actions (17).

IL-3 exerts its biological activities through binding to heteromeric transmembrane receptors consisting of a (IL-3 Rα) and common β (β_c) subunits. The β_c subunit is shared with IL-5 and GM-CSF, while the IL-3 Rα subunit is specific for IL-3 (18). The IL-3 Rα subunit is a low affinity IL-3 receptor but upon association with the β_c subunit forms the functional high affinity receptor complex. In mice, a second IL-3-specific β subunit has been identified and termed βIL-3 (19).

The βc subunit represents the primary signal-transducing element of the IL-3 receptor (18). However, variations in signal transduction in the same cell system via IL-3, GM-CSF, and IL-5, and altered responses from cells transfected with mutant α subunits suggests that the α subunit plays a role in signaling as well (20-22). Neither the IL-3 Rα nor the β_c subunit contains intrinsic kinase activity; however, upon ligand binding and heterodimerization (23, 24), the receptor is phosphorylated on multiple residues (25, 26). In a variety of murine and human cell types, the activated receptor can then interact with directly, or lead to the context-dependent activation of many effector molecules including JAKs (27, 28), STATs (29, 30), PI-3 kinase (31), AKT (32), NF-κB (31), Src (33), Rac (34), Ras (35), and MAP kinases (32).

IL-3 has documented activity *in vitro* in both hematopoietic and non-hematopoietic systems. It has been shown that IL-3 alone, or acting synergistically with other early acting hematopoietic growth factors including, IL-1, IL-6, stem cell factor, G-CSF, Thrombopoietin, and Flt-3 Ligand, can support the proliferation of pluripotent hematopoietic stem cells and progenitor cells of various lineages (36-40). IL-3 is often a part of cytokine cocktails used to promote *ex vivo* expansion of human $CD34^+$ hematopoietic stem cells (38, 39), although some studies suggest that IL-3 may inhibit the repopulating potential of these cells (41-43). In addition to its effects on hematopoietic cells, IL-3 can stimulate human endothelial (29, 44) and smooth muscle cell (45) proliferation and motility. IL-3 has also been shown to act as a neurotrophic factor with the ability to promote neuronal survival and enhance neurite outgrowth (46-49). The roles of IL-3 have been assessed *in vivo* using knockout mice and yet remain unclear. Despite the many effects of IL-3 *in vitro*, IL-3-deficient mice are viable and exhibit outwardly normal hematopoiesis (50, 51). IL-$3^{-/-}$ mice do appear to exhibit deficiencies in mast cell and basophil development in addition to compromised immunity to parasites (52) and reductions in delayed hypersensitivity reactions (53).

The Quantikine® Human IL-3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-3 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-3 and antibodies raised against the recombinant factor. Natural human IL-3 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standard. These results indicate that this kit can be used to determine relative levels of natural human IL-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-3 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-3 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 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-3 Microplate	890009	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-3.	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-3 Standard	890011	Recombinant human IL-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human IL-3 Conjugate	890010	21 mL of a polyclonal antibody specific for human IL-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-6	895158	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use. For serum/plasma samples.*	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservative. *For cell culture supernate samples.*	
Calibrator Diluent RD6-35	895360	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
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	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.
- Test tubes for dilution of standards.
- Human IL-3 Controls (optional; R&D Systems®, Catalog # QC21).

PRECAUTIONS

Some components of this kit contain 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, 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

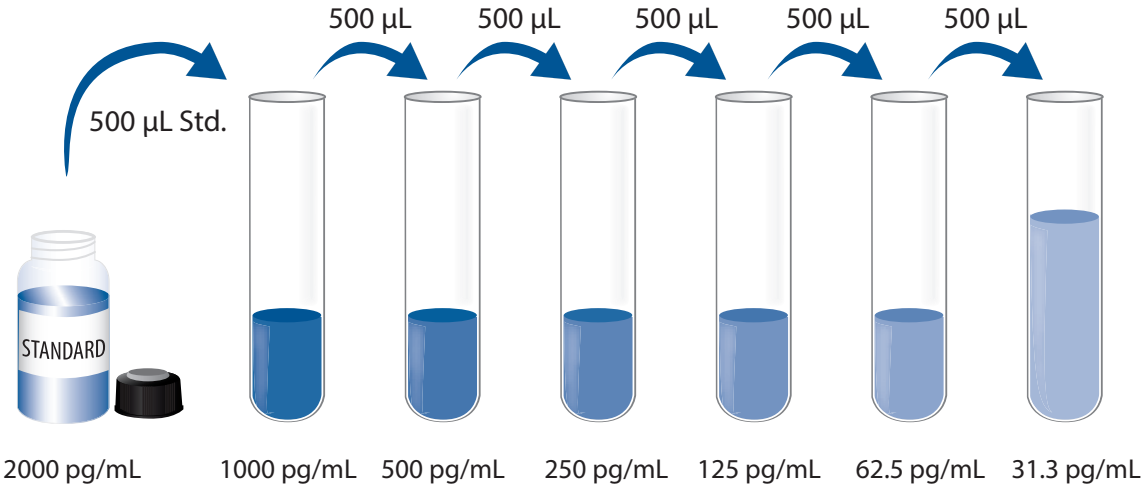
Bring all reagents to room temperature before use.

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.

Human IL-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-3 Standard with Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-35 (*for serum/plasma samples)*. This reconstitution produces a stock solution of 2,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-35 (*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 Human IL-3 Standard (2000 pg/mL) 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. **For Serum/Plasma Samples Only:** Add 50 µL of Assay Diluent RD1-6 to each well. *Assay Diluent RD1-6 may have a precipitate present. Mix well before and during use.*
4. Add 200 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 the standard 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-3 Conjugate to each well. Cover with a new adhesive strip. 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 20 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 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.).

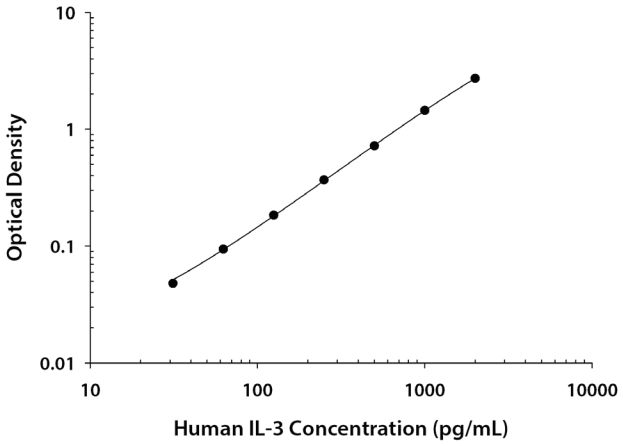
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-3 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

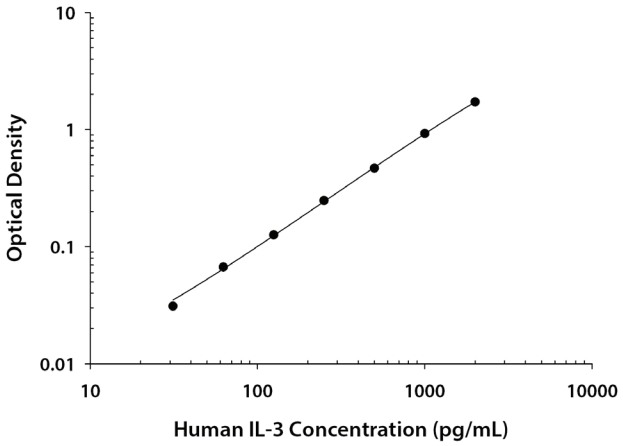
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.017	0.017	—
31.3	0.065 0.064	0.064	0.047
62.5	0.113 0.108	0.110	0.093
125	0.204 0.198	0.201	0.184
250	0.386 0.384	0.385	0.368
500	0.749 0.727	0.738	0.721
1000	1.489 1.438	1.464	1.447
2000	2.741 2.732	2.736	2.719

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.023	0.025	—
31.3	0.055 0.057	0.056	0.031
62.5	0.092 0.093	0.092	0.067
125	0.150 0.153	0.152	0.127
250	0.275 0.271	0.273	0.248
500	0.496 0.491	0.494	0.469
1000	0.944 0.954	0.949	0.924
2000	1.711 1.784	1.748	1.723

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 tweny 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)	126	731	991	128	725	1423
Standard deviation	5.70	23.7	30.4	10.1	45.9	81.5
CV (%)	4.5	3.2	3.1	7.9	6.3	5.7

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)	115	728	1460	124	737	1450
Standard deviation	6.50	23.6	48.7	13.3	41.4	104
CV (%)	5.7	3.2	3.3	10.7	5.6	7.2

RECOVERY

The recovery of human IL-3 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	92	78-105%
Serum	104	96-118%
EDTA plasma	108	96-117%
Heparin plasma	100	93-108%
Citrate plasma	104	93-122%

LINEARITY

To assess linearity of the assay, the following samples spiked with high concentrations of human IL-3 were diluted with the appropriate calibrator diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture media	Neat	564	———	———
	1:2	312	282	111
	1:4	154	141	109
	1:8	71	71	100
Serum	Neat	846	———	———
	1:2	382	423	90
	1:4	179	212	84
	1:8	86	106	81
	1:16	45	53	85
EDTA plasma	Neat	951	———	———
	1:2	461	476	97
	1:4	223	238	94
	1:8	111	119	93
	1:16	54	59	92
Heparin plasma	Neat	708	———	———
	1:2	371	354	105
	1:4	190	177	107
	1:8	97	88	110
	1:16	48	44	109
Citrate plasma	Neat	849	———	———
	1:2	396	424	93
	1:4	196	212	92
	1:8	101	106	95
	1:16	50	53	94

SENSITIVITY

The minimum detectable dose (MDD) of human IL-3 is typically less than 7.4 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-3 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Forty-nine serum samples from apparently healthy volunteers were evaluated for the presence of human IL-3 in this assay. All samples measured less than the lowest Human IL-3 Standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

SPECIFICITY

This assay recognizes natural and recombinant human IL-3.

The factors listed below were prepared at 10 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 10 ng/mL in a mid-range recombinant human IL-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF
GM-CSF
IL-1α
IL-1β
IL-2
IL-3 Rα
IL-3 Rβ
IL-4
IL-6
IL-7
IL-8
LIF
TGF-β1
TNF-α
TNF-β

Recombinant mouse:

GM-CSF
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7

Natural proteins:

human TGF-β1

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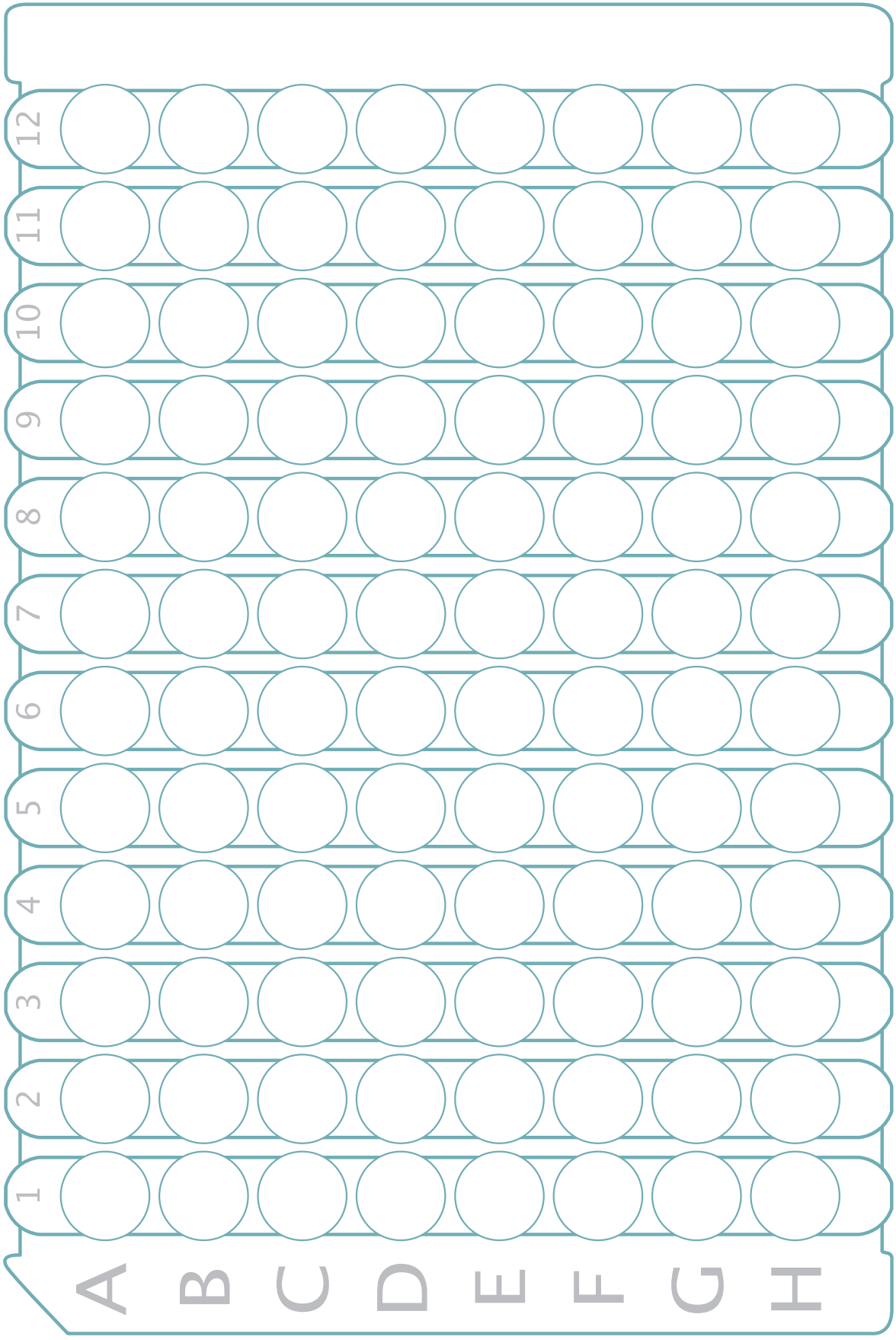
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES