Quantikine® ELISA

Human IL-17 Immunoassay

Catalog Number D1700
S1700
PD1700

For the quantitative determination of human Interleukin 17 (IL-17) 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

Human Interleukin 17 (IL-17), also known as IL-17A and CTLA-8, is a 15-20 kDa, variably glycosylated polypeptide that belongs to the IL-17 family of cytokines (1-5). Its alternate name, CTLA-8, originated from rodent studies where an activated hybridoma was created from the fusion of a mouse cytotoxic and a rat T cell lymphoma cell line. The molecule of interest in this study was assumed to have come from the mouse cytotoxic lymphocyte cell (thus the CTL designation), whereas, in fact, it was a rat lymphocyte molecule. Human IL-17/17A is synthesized as a 155 amino acid (aa) precursor that contains a 23 aa signal sequence and a 133 aa mature region that possesses a cysteine-knot fold (4-6). In both human and mouse, there is one conserved N-linked glycosylation site that likely contributes 5 kDa to its native molecular weight. IL-17A forms both a 32-38 kDa disulfide-linked homodimer, and a 40-45 kDa covalent heterodimer with IL-17F (7-9). Most secreted IL-17A is in the form of the IL-17A:F heterodimer, however, the IL-17A:A homodimer is the most bioactive of the two forms (8). Mature human IL-17A is 61%, 74%, and 99% aa identical to mouse, porcine, and chimpanzee IL-17A, respectively (10-12). Mammalian cells known to produce IL-17 are the $CD4^+$ Th17 T cells, Paneth cells, $GR1^+CD11b^+$ myeloid suppressor cells, $CD27^-\gamma\delta$ T cells, $CD1^+NK1.1^-$iNKT cells, and $CD3^-CD4^+$ LTi-like cells (9, 13-17).

A high affinity receptor for human IL-17 has been reported, and appears to be a heteromultimer of IL-17RA and IL-17RC, likely in a 2:1 ratio (1, 18). IL-17RA is a 130 kDa, type I transmembrane glycoprotein that bears no resemblance to members of the cytokine, TNF or immunoglobulin receptor superfamily (2, 10, 15). IL-17RC is also a type I transmembrane protein, approximately 90-95 kDa in size, that shares less than 30% aa identity with IL-17RA (19, 20). Both receptors are needed for IL-17A and IL-17A:F activity. The two receptors appear to form a functional association following ligand binding to IL-17RA (1, 21, 22).

IL-17 is best known for its participation in the recruitment and survival of neutrophils (14, 15, 23, 24, 25). Its induction was initially described to be the result of antigen stimulation of dendritic cells, resulting in IL-23 secretion. In a T cell receptor-independent event, IL-23 induces T cell production of IL-17 (14). Once secreted, IL-17 in the bone marrow would seem to induce stromal/fibroblast expression of both G-CSF and stem cell factor (membrane form), an effect that increases polymorphonuclear neutrophils (PMN) differentiation and production. IL-17 may complement this by directly blocking neutrophil apoptosis, promoting greater circulating PMN numbers (23). In the tissues, IL-17 would also seem to promote neutrophil extravasation, principally through its effects on macrophages and endothelial cells (EC). On macrophages, IL-17 induces TNF-α, IL-1β and IL-6 production (26). TNF-α and IL-1β then act on local ECs to induce G-CSF secretion, an effect that is potentiated by IL-17 (27). IL-17 further contributes to PMN influx by inducing EC CXC chemokine release and NO production, which may increase vascular permeability (14, 28). IL-17 effects are not limited to inflammation. In synovial joints, IL-17 upregulates RANKL expression on osteoblasts. This provides a stimulus for osteoclast formation and subsequent bone resorption (24). In conjunction with IL-4 and CD40L, IL-17A also promotes the generation of IgE secreting cells (29). And in white fat, IL-17A inhibits adipocyte differentiation from preadipocytes, and impairs glucose uptake by mature adipocytes (30).

The Quantikine® Human IL-17 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-17 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed human IL-17 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human IL-17 accurately. Results obtained using natural human IL-17 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-17.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-17 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-17 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-17 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-17 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.
- 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 # D1700	CATALOG # S1700	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-17 Microplate	890579	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-17.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-17 Conjugate	890580	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-17 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-17 Standard	890581	1 vial	6 vials	Recombinant human IL-17 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-36	895272	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5R	895190	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-21	895261	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 preservatives. *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.

D1700 contains sufficient materials to run an ELISA on one 96 well plate.
S1700 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD1700). 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-17 Controls (optional; R&D Systems®, Catalog # QC21).

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

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 citrate, 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: *Grossly hemolyzed samples are not suitable for use in this assay.*

REAGENT PREPARATION

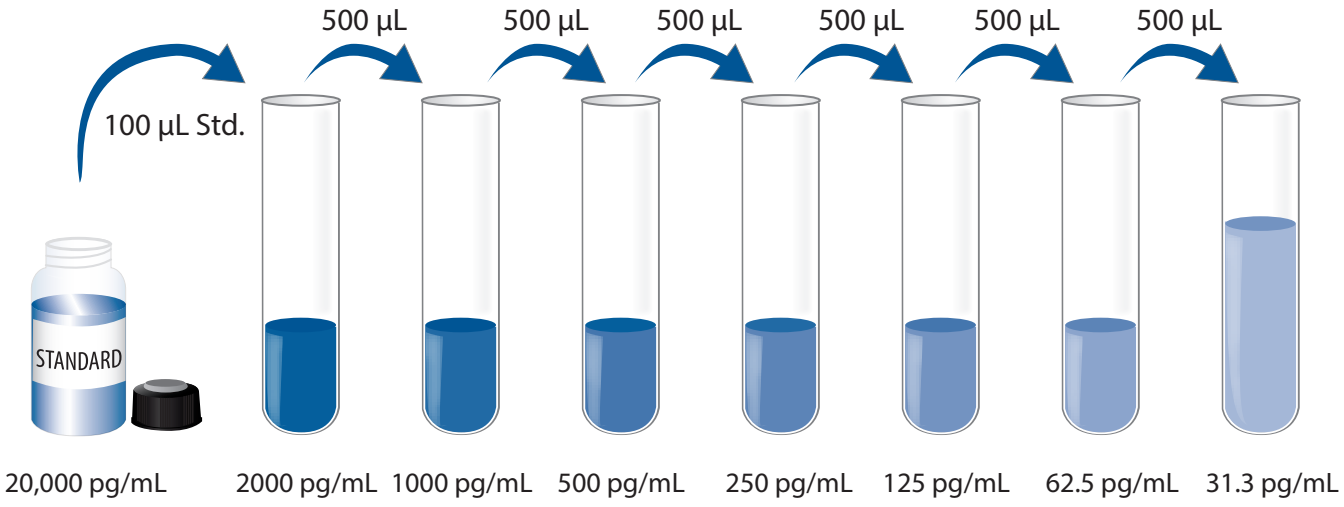
Bring all reagents to room temperature before use.

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-17 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-17 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.

Pipette 900 µL of the Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-21 (*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 samples, controls, and standards 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 RD1-36 to each well.
4. Add 100 µL of standard, control, or sample per well. Ensure reagent addition is uninterrupted and completed **within 15 minutes.** Cover with the adhesive strip provided. Incubate 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 twice for a total of three 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-17 Conjugate to each well. Cover with a new adhesive strip. 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.).

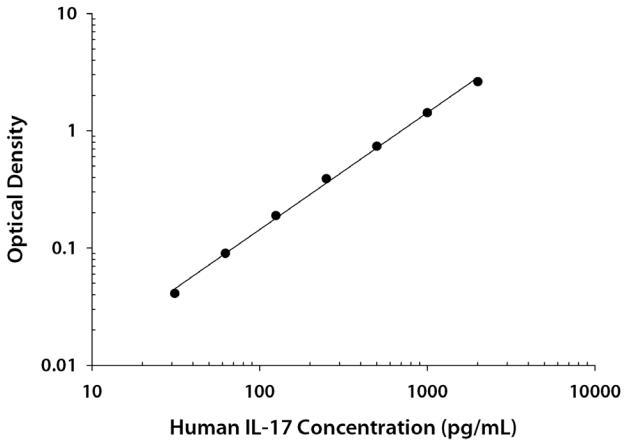
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-17 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

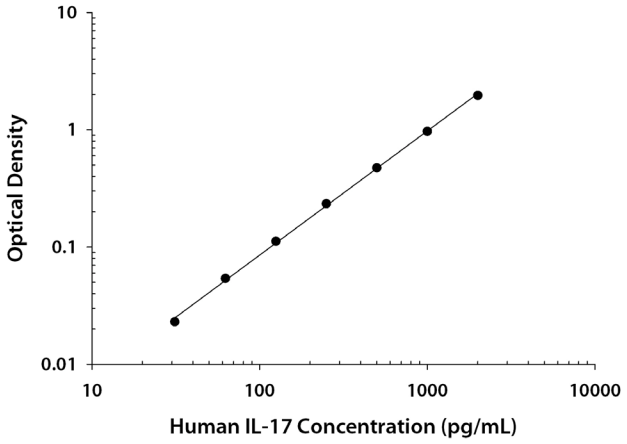
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.057 0.055	0.056	—
31.3	0.100 0.094	0.097	0.041
62.5	0.149 0.142	0.146	0.090
125	0.252 0.238	0.245	0.189
250	0.459 0.435	0.447	0.391
500	0.776 0.813	0.794	0.738
1000	1.465 1.495	1.480	1.424
2000	2.673 2.692	2.682	2.626

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.042 0.047	0.044	—
31.3	0.067 0.068	0.067	0.023
62.5	0.097 0.099	0.098	0.054
125	0.154 0.159	0.156	0.112
250	0.277 0.280	0.278	0.234
500	0.512 0.522	0.517	0.473
1000	1.003 1.023	1.013	0.969
2000	1.930 2.090	2.010	1.966

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)	107	604	1217	76.6	272	549
Standard deviation	7.8	25.0	63.3	6.5	23.4	43.9
CV (%)	7.3	4.1	5.2	8.5	8.6	8.0

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)	131	736	1480	125	408	794
Standard deviation	5.8	34.6	61.2	9.8	34.3	55.6
CV (%)	4.4	4.7	4.1	7.8	8.4	7.0

RECOVERY

The recovery of human IL-17 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	97	88-112%
Serum (n=5)	98	90-105%
Citrate plasma (n=5)	105	92-116%
EDTA plasma (n=5)	101	90-110%
Heparin plasma (n=5)	101	93-109%

LINEARITY

To assess the linearity of the assay, five samples spiked with high concentrations of human IL-17 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	Citrate plasma (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	100	96	93	91	92
	Range (%)	97-103	93-100	91-97	88-95	86-96
1:4	Average % of Expected	100	97	97	94	91
	Range (%)	91-107	95-100	92-105	86-102	85-107
1:8	Average % of Expected	95	100	103	97	94
	Range (%)	82-106	86-107	92-109	82-107	86-106
1:16	Average % of Expected	101	101	101	95	100
	Range (%)	87-114	96-108	95-112	82-110	86-108

SENSITIVITY

The minimum detectable dose (MDD) of human IL-17 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 *E. coli*-expressed recombinant human IL-17 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Thirty-four samples from apparently healthy volunteers were evaluated for the presence of human IL-17 in this assay. All samples measured less than the lowest standard, 31.3 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 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were 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-17.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	52.2
Stimulated	54.9	110

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-17.

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-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-17B
IL-17B R
IL-17C
IL-17D
IL-17E
IL-17F
IL-17 R
IL-17 RC
IL-17 RD

Recombinant mouse:

IL-17
IL-17A
IL-17B
IL-17B R
IL-17C
IL-17D
IL-17E
IL-17F
IL-17 RD
IL-17 RE

Recombinant human IL-17 A/F Heterodimer cross-reacts approximately 10.3% in this assay.

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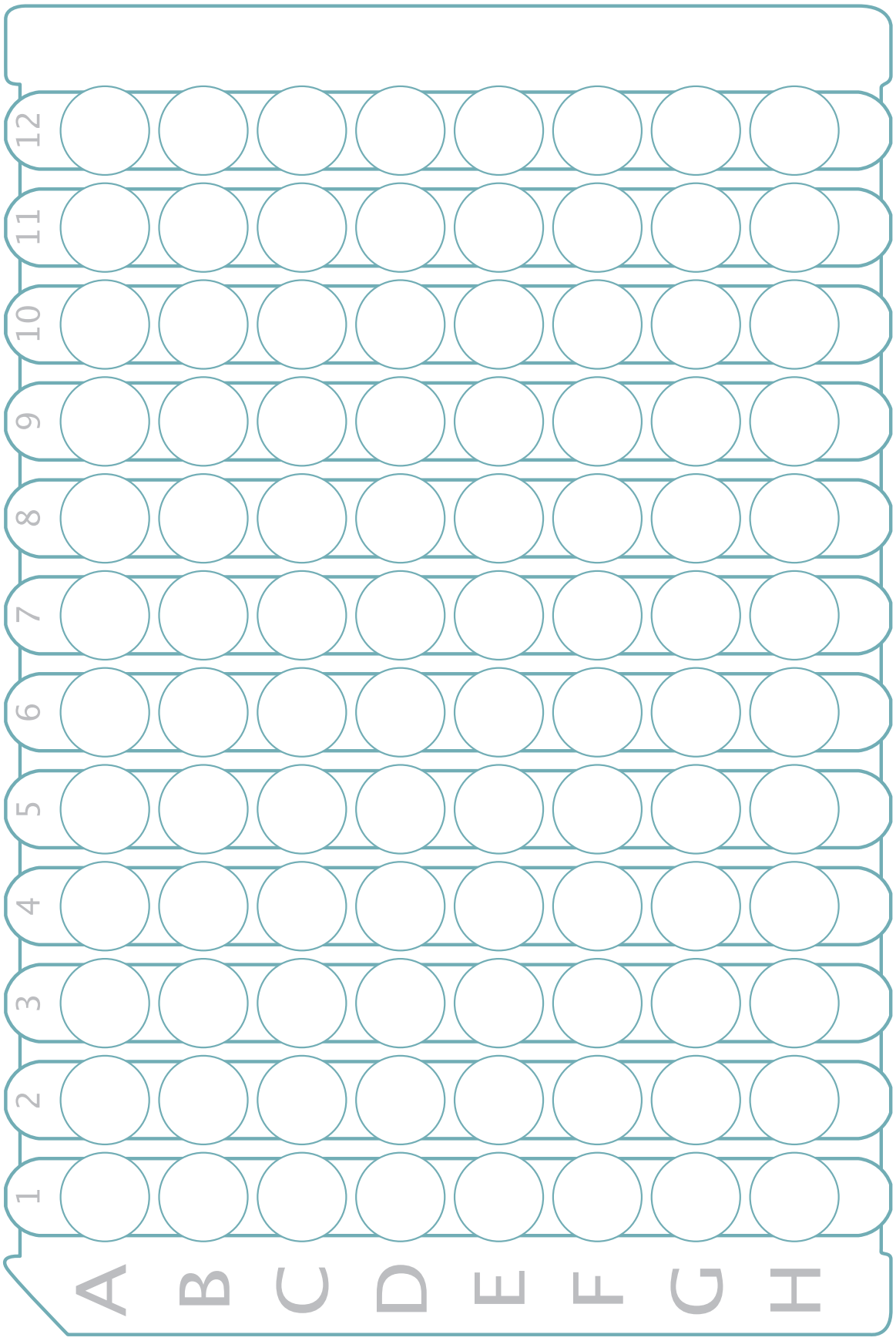
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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