Quantikine® ELISA

Human IL-20 Immunoassay

Catalog Number DL200

For the quantitative determination of human Interleukin 20 (IL-20) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. 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

Human Interleukin 20 (IL-20) is a member of the IL-10 family of cytokines (1). It exhibits approximately 28% amino acid (aa) identity with IL-10 and 76% aa identity with mouse IL-20 (2, 3). It was identified by searching databases for translated sequences containing a signal sequence and amphipathic helices found in helical cytokines (2). Human IL-20 is synthesized as a 176 aa precursor with a 24 aa signal sequence and a 152 aa mature segment (2). IL-20 appears to function as a monomer (2, 4). Expression of IL-20 can be upregulated by treatment with lipopolysaccharide (5).

There are two heterodimeric receptor complexes for IL-20 (6, 7). The first is composed of IL-20 Rα and IL-20 Rβ. The second is composed of IL-22 R and IL-20 Rβ. Whereas the IL-22 R/IL-20 Rβ complex is shared with IL-24, the IL-20 Rα/IL-20 Rβ complex is shared with both IL-19 and IL-24 (8). IL-20 has been shown to initiate transduction cascades involving STAT3 and stimulates the induction of pro-inflammatory genes including TNF-α and MCP-1 (2, 9).

Initial functional studies using transgenic mice suggest that IL-20 has the ability to regulate skin development (2). The over-expression of both human and mouse forms of IL-20 results in keratinocyte hyperproliferation, abnormal epidermal differentiation, and neonatal lethality (2). In humans, IL-20 and its receptors are upregulated in psoriatic skin, and polymorphisms in the IL-20 gene have been associated with plaque-type psoriasis (2, 10, 11). IL-20 may also have a role in hematopoiesis. It enhances the proliferation of multipotential progenitors *in vitro* and increases their numbers and cell cycling status in IL-20 transgenic mice (12). IL-20 is also shown to suppress COX-2 and PGE2 and acts as an inhibitor of angiogenesis in model systems (13).

The Quantikine® Human IL-20 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IL-20 in cell culture supernates, serum, and plasma. It contains *E. coli*- expressed recombinant human IL-20 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-20 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-20.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-20 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-20 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-20 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-20 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, 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-20 Microplate	892979	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-20.	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-20 Standard	892981	Recombinant human IL-20 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 1 month.*
Human IL-20 Conjugate	892980	21 mL of a polyclonal antibody specific for human IL-20 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:10 for cell culture supernate samples. Use diluted 1:2.5 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	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.
- Test tubes for dilution of standards.
- Human IL-20 Controls (optional; R&D Systems®, Catalog # QC28).

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 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 has not been validated 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.

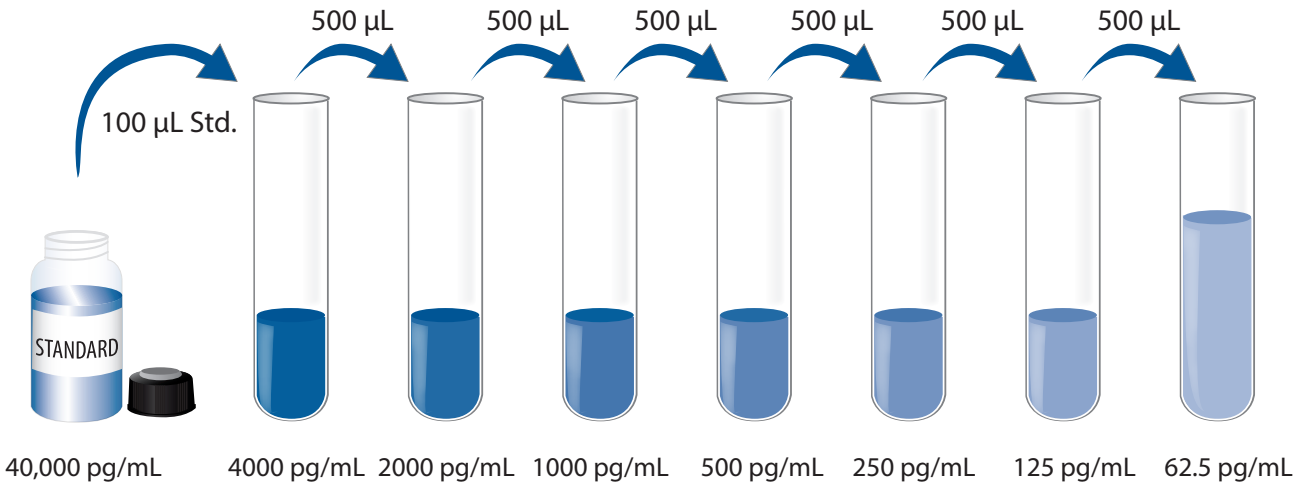
Calibrator Diluent RD5P (diluted 1:10) - **For cell culture supernate samples only.** Add 1.0 mL of Calibrator Diluent RD5P Concentrate to 9.0 mL of deionized or distilled water to prepare 10 mL of Calibrator Diluent RD5P (diluted 1:10).

Calibrator Diluent RD5P (diluted 1:2.5) - **For serum/plasma samples only.** Add 4.0 mL of Calibrator Diluent RD5P Concentrate to 6.0 mL of deionized or distilled water to prepare 10 mL of Calibrator Diluent RD5P (diluted 1:2.5).

Note: *Prepare only as much calibrator diluent as needed per day. Discard after use.*

Human IL-20 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-20 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:10) (*for cell culture supernate samples*) or Calibrator Diluent RD5P (diluted 1:2.5) (*for serum/plasma samples*) into the 4000 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 4000 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 RD1W 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. 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-20 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 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.).

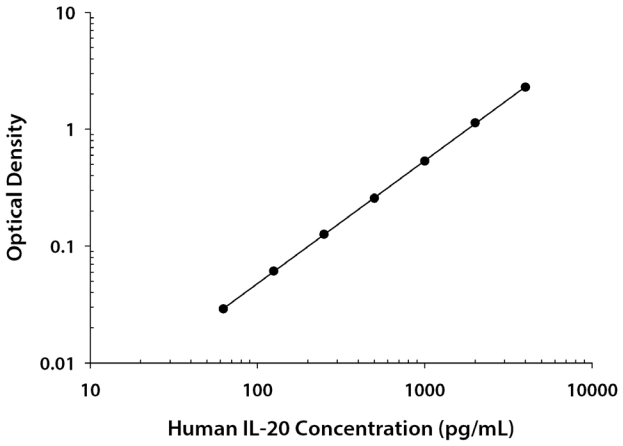
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-20 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

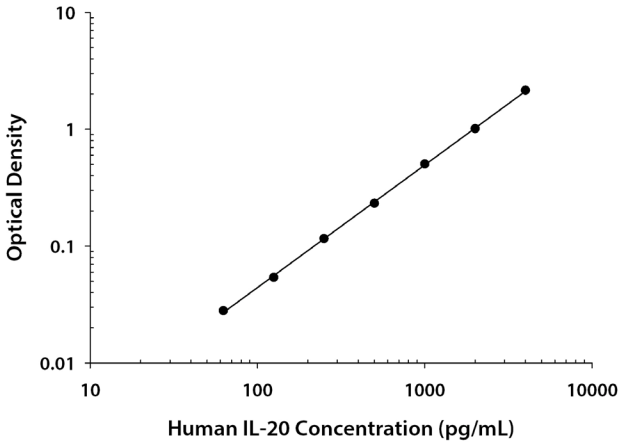
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.020	0.019	—
62.5	0.047 0.049	0.048	0.029
125	0.079 0.081	0.080	0.061
250	0.144 0.146	0.145	0.126
500	0.272 0.279	0.276	0.257
1000	0.552 0.553	0.553	0.534
2000	1.154 1.155	1.155	1.136
4000	2.292 2.318	2.305	2.286

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.018	0.018	—
62.5	0.045 0.046	0.046	0.028
125	0.070 0.074	0.072	0.054
250	0.131 0.136	0.134	0.116
500	0.247 0.252	0.250	0.232
1000	0.521 0.522	0.522	0.504
2000	1.027 1.029	1.028	1.010
4000	2.149 2.189	2.169	2.151

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)	461	984	1983	491	961	1882
Standard deviation	26.1	70.4	159	39.8	74.2	106
CV (%)	5.7	7.2	8.0	8.1	7.7	5.6

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)	522	1143	2183	518	1079	2092
Standard deviation	32.2	103	208	46.4	61.8	101
CV (%)	6.2	9.0	9.5	9.0	5.7	4.8

RECOVERY

The recovery of human IL-20 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	96-111%
Serum (n=4)	100	91-109%
EDTA plasma (n=4)	107	103-113%
Heparin plasma (n=4)	101	86-108%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-20 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	97	100	92	92
	Range (%)	94-98	97-103	91-95	88-93
1:4	Average % of Expected	94	99	92	96
	Range (%)	92-96	94-106	91-94	94-97
1:8	Average % of Expected	96	100	97	96
	Range (%)	93-99	97-103	90-102	93-99
1:16	Average % of Expected	100	100	99	101
	Range (%)	98-103	99-103	94-104	95-114

SENSITIVITY

One hundred one assays were evaluated and the minimum detectable dose (MDD) of human IL-20 ranged from 2.63-16.6 pg/mL. The mean MDD was 7.51 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-20 manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-20 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=36)	68.7	2.8	ND-68.7
EDTA plasma (n=36)	68.7	2.8	ND-68.7
Heparin plasma (n=36)	71.0	2.8	ND-71.0

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 5.0 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of human IL-20. No detectable levels were observed.

Human monocytes (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, enriched with Percoll gradient (PBMC isolated with Ficoll). Cells were cultured unstimulated or stimulated with 100 ng/mL LPS. Aliquots of the cell culture supernate were removed and assayed for levels of human IL-20.

Condition	Culture 1 (pg/mL)	Culture 2 (pg/mL)
Unstimulated	ND	ND
Stimulated	73.6	120

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-20.

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-20 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CD40
CD40 Ligand
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-15
IL-16
IL-17
IL-19
IL-20 Rα
IL-20 Rβ
IL-22
IL-24
IL-26 (monomer)
IL-26 (dimer)
IL-28A
IL-29
TNF-α

Recombinant mouse:

IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-17
IL-20
TNF-α

Recombinant rat:

IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
TNF-α

Recombinant porcine:

IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-8
IL-10
TNF-α

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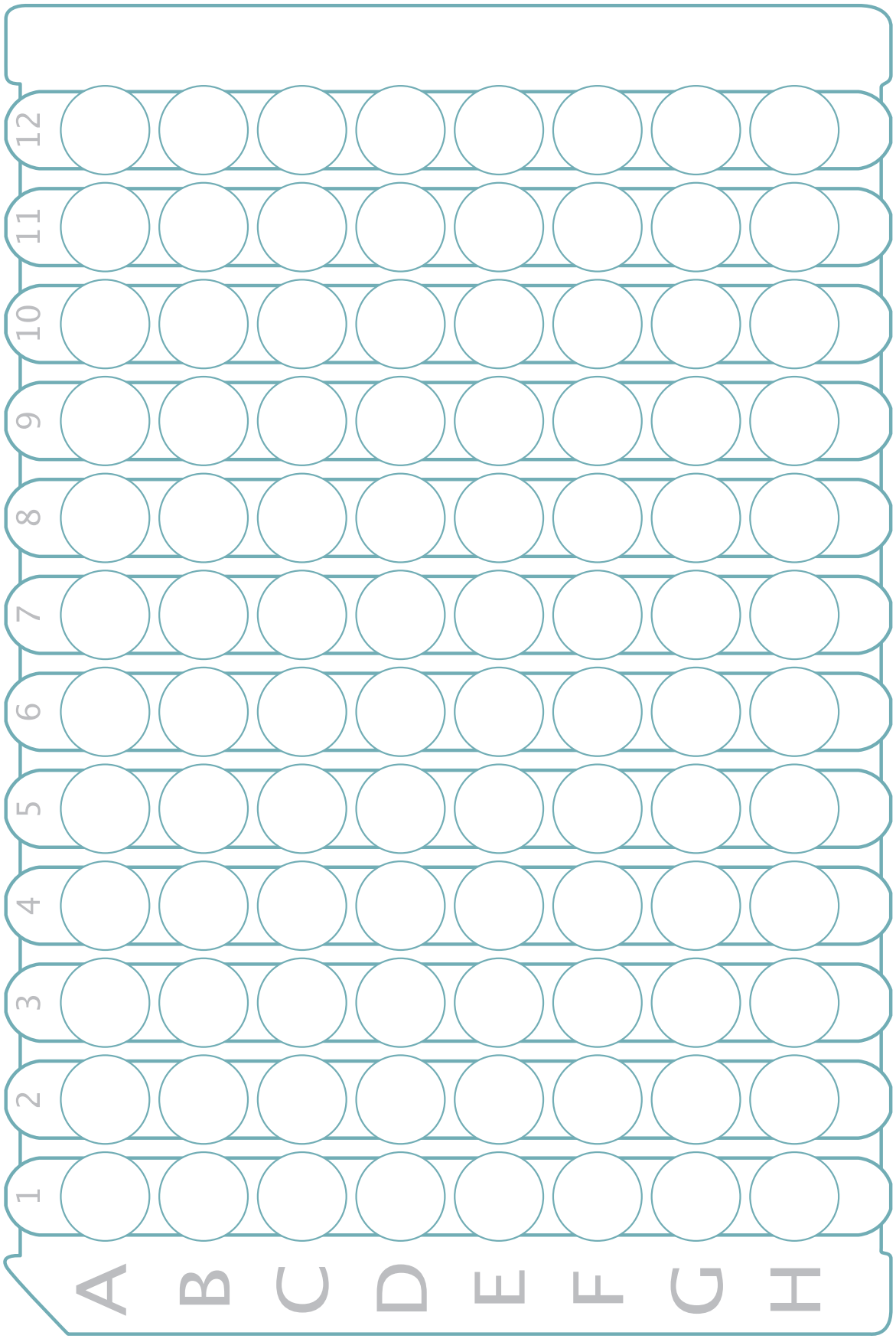
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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