Quantikine® ELISA

Mouse IL-13 Rα2 Immunoassay

Catalog Number M13RA2

For the quantitative determination of mouse Interleukin 13 Receptor alpha 2 (IL-13 Rα2) concentrations in cell culture supernates, tissue homogenates, 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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USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Interleukin-13 Receptor alpha 2 (IL-13 Rα2), also known as IL-13 binding protein and CD213a2, is a widely expressed 55 kDa cytokine receptor that plays an important role in the Th2-polarized immune responses characteristic of a variety of pathologies including parasitic infections and allergic asthma (1, 2). Mature mouse IL-13 Rα2 consists of a 313 amino acid (aa) extracellular domain with three fibronectin type-III domains, a WSxWS motif, a 21 aa transmembrane segment, and a 28 aa cytoplasmic domain (3). Within the ECD, mouse IL-13 Rα2 shares 64% and 94% aa sequence identity with human and rat IL-13 Rα2, respectively. A 40-50 kDa soluble form of mouse IL-13 Rα2 can be generated by alternate splicing or MMP-8 mediated shedding *in vitro* (4-6). In humans, soluble IL-13 Rα2 is likely only generated by shedding, since a soluble splice form has not been described (7). Production of the soluble receptor can be induced in human bronchial epithelial cells by IL-13 or lysophosphatidic acid stimulation (8, 9). House dust mite allergen induces the release of IL-13 Rα2 into mouse bronchoalveolar lavage fluid (BALF) (10). In contrast to mouse, soluble human IL-13 Rα2 is only weakly released into plasma or BALF (7). IL-13 Rα2 is upregulated in the myocardium during heart failure (11), on pulmonary arterial smooth muscle during pulmonary hypertension (12), and on intestinal epithelial cells in ulcerative colitis and colorectal cancer (13).

The biological effects of IL-13 and IL-4 are closely related due in part to a shared receptor system. IL-13 binds to IL-13 Rα1 which then forms a signaling complex with IL-4 Rα (14, 15). IL-13 Rα2 functions as a decoy receptor by binding and internalizing IL-13 and preventing it from signaling through the IL-13 Rα1/IL-4 Rα complex (6, 16). IL-13 Rα2 can also block IL-4 induced responses even though it does not itself bind IL-4. Instead, it inhibits signaling through IL-13 Rα1/IL-4 Rα receptor complexes which have already bound IL-4 (17, 18). Soluble IL-13 Rα2 retains ligand binding capability and attenuates responses to IL-13 but not to IL-4 (17, 19). Transmembrane as well as soluble IL-13 Rα2 are upregulated during Th2-biased immune responses and limit the extent of those responses (20-23). IL-13 Rα2 promotes tumorigenesis and metastasis by its ability to block IL-13 and IL-4 (18, 24, 25). Aside from its decoy function, IL-13 Rα2 can signal in response to IL-13 to directly promote tumor cell invasiveness and the development of tissue fibrosis (25, 26).

The Quantikine® Mouse IL-13 Rα2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-13 Rα2 levels in cell culture supernates, tissue homogenates, serum, and plasma. It contains NS0-expressed recombinant mouse IL-13 Rα2 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-13 Rα2 accurately. Results obtained using natural mouse IL-13 Rα2 showed dose-response 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 mouse IL-13 Rα2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-13 Rα2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-13 Rα2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-13 Rα2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-13 Rα2 bound in the initial step. The sample values are then read off the standard curve.

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 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.
- 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.

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
Mouse IL-13 Rα2 Microplate	894257	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-13 Rα2.	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.*
Mouse IL-13 Rα2 Conjugate	894258	12 mL of a polyclonal antibody specific for mouse IL-13 Rα2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse IL-13 Rα2 Standard	894259	Recombinant mouse IL-13 Rα2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse IL-13 Rα2 Control	894260	Recombinant mouse IL-13 Rα2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated 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	895174	23 mL of diluted hydrochloric 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.
- 100 mL and 1000 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

If using tissue homogenate samples, the following are also required:

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).
- PBS

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Prior to assay, tissues must be homogenized according to the directions in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 20 minutes at 2000 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.*

SAMPLE PREPARATION

Tissue homogenate samples require a 4-fold dilution. A suggested 4-fold dilution is 40 µL of sample + 120 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IL-13 Rα2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

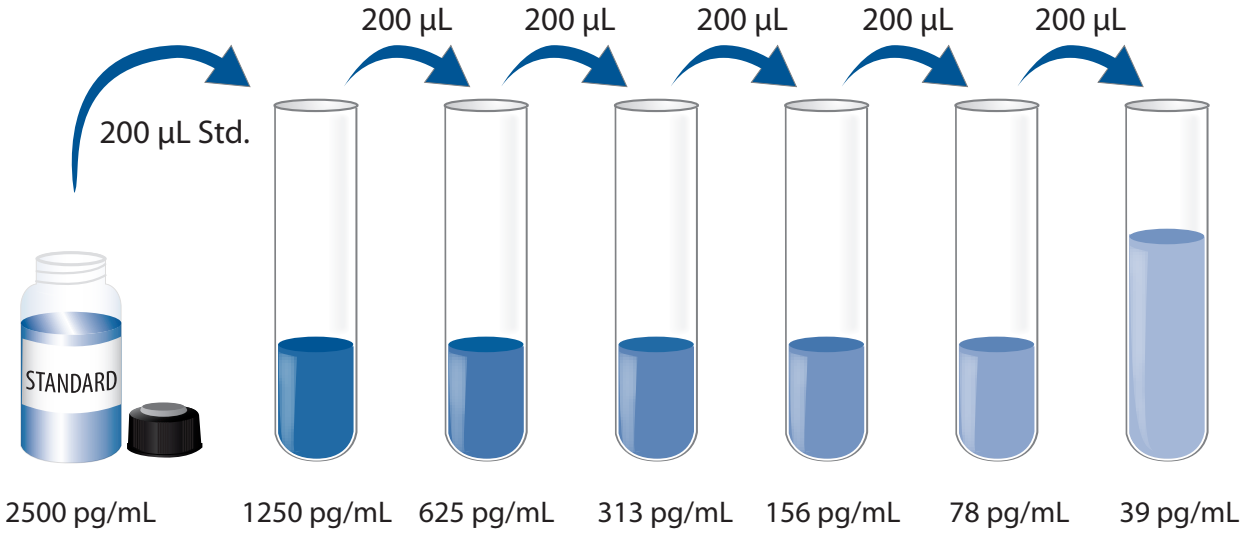
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Mouse IL-13 Rα2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-13 Rα2 Standard with Calibrator Diluent RD5P (diluted 1:5). This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-13 Rα2 Standard (2500 pg/mL) serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, 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 RD1W to each well.
4. Add 50 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 the 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 100 µL of Mouse IL-13 Rα2 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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

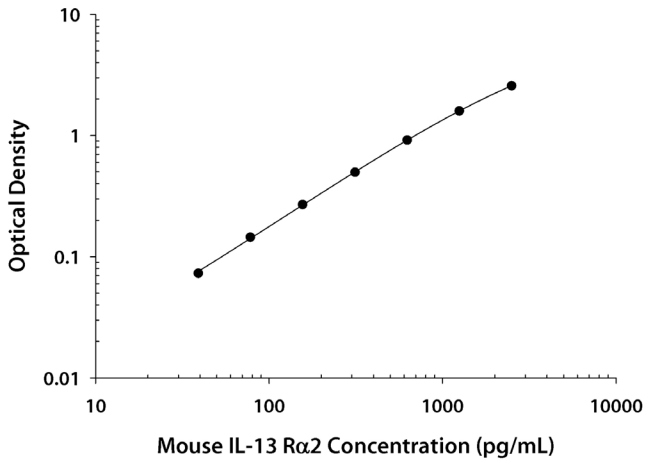
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse IL-13 Rα2 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	0.017 0.018	0.018	—
39	0.090 0.092	0.091	0.073
78	0.161 0.165	0.163	0.145
156	0.286 0.288	0.287	0.269
313	0.513 0.517	0.515	0.497
625	0.930 0.942	0.936	0.918
1250	1.594 1.629	1.612	1.594
2500	2.566 2.614	2.590	2.572

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)	97.7	338	1011	101	279	1008
Standard deviation	4.25	12.5	23.2	8.2	17.6	50.3
CV (%)	4.4	3.7	2.3	8.1	6.3	5.0

RECOVERY

The recovery of mouse IL-13 Rα2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	91	83-98%
Tissue homogenates* (n=4)	106	93-119%
Serum* (n=4)	97	83-108%
EDTA plasma* (n=4)	99	97-101%
Heparin plasma* (n=4)	103	87-117%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-13 Rα2 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Tissue homogenates* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	104	101	101	101	103
	Range (%)	101-105	92-108	98-103	94-105	101-105
1:4	Average % of Expected	106	102	106	104	105
	Range (%)	100-115	90-115	103-110	96-110	99-112
1:8	Average % of Expected	107	105	108	105	105
	Range (%)	101-110	94-115	107-109	94-114	98-112
1:16	Average % of Expected	110	113	110	105	101
	Range (%)	102-116	110-115	108-112	95-115	98-105

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse IL-13 Rα2 ranged from 1.0-5.6 pg/mL. The mean MDD was 2.3 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 NS0-derived recombinant mouse IL-13 Rα2 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-13 Rα2 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	14,454	9660-25,460	4626
Plasma (n=10)	15,062	7720-21,440	4229

Cell Culture Supernates - LL/2 Mouse Lewis lung carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, and stimulated with 1 µg/mL lipopolysaccharide for 4 days. An aliquot of the cell culture supernate was removed, assayed for mouse IL-13 Rα2, and measured 243 pg/mL.

Tissue Homogenates - Organs from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the homogenates were removed and assayed for levels of mouse IL-13 Rα2.

Tissue Type	(pg/mL)
Brain	422
Heart	233
Kidney	804
Liver	2472
Lung	345
Spleen	1761

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-13 Rα2.

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 mouse IL-13 Rα2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-4
IL-4 R
IL-13 Rα1

Recombinant human:

IL-4
IL-4 R
IL-5 Rα
IL-9 R
IL-13 Rα1
IL-13 Rα2

Recombinant mouse, rat, and human IL-13 does not cross-react in this assay but does interfere at concentrations > 1000 pg/mL.

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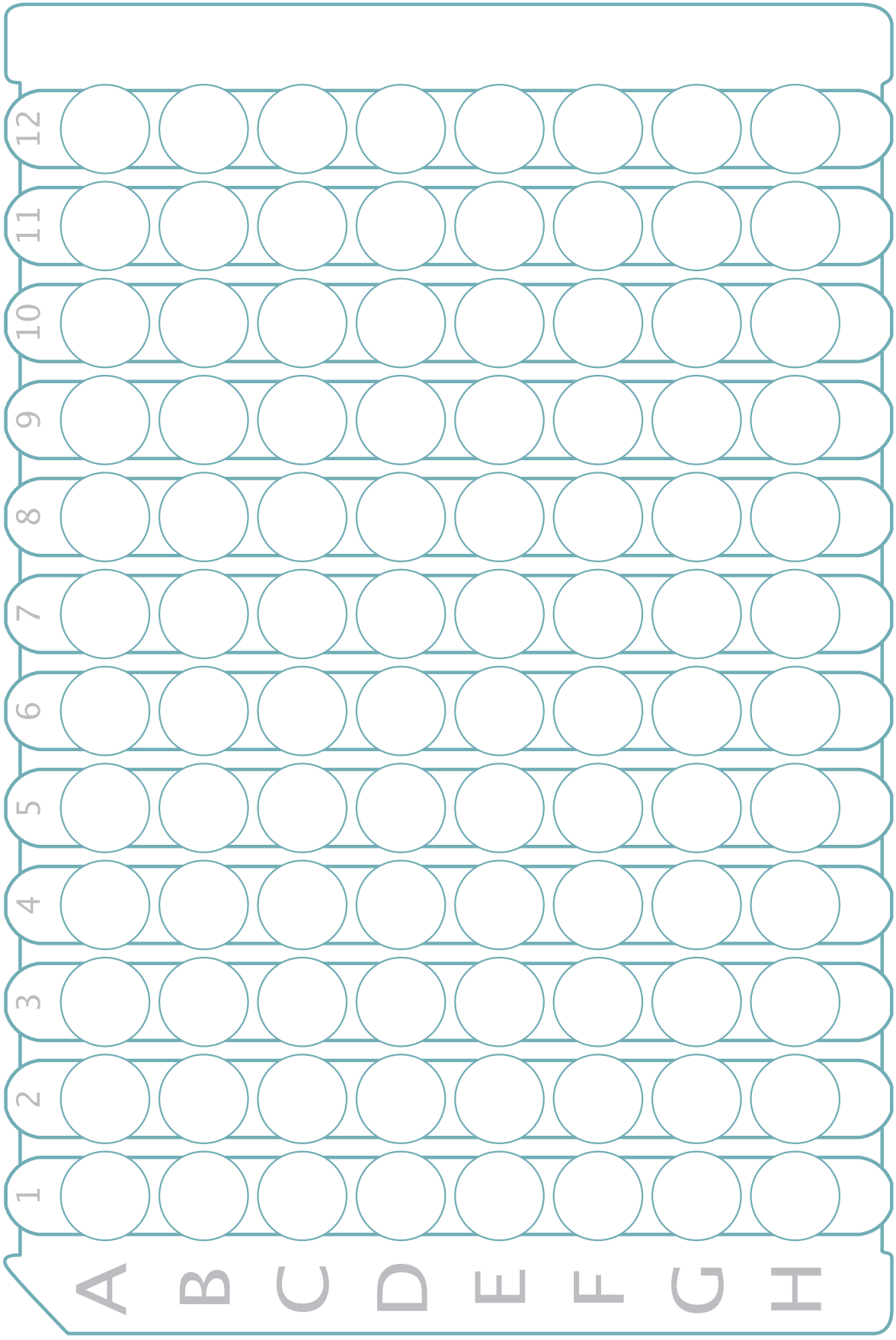
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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