Quantikine® ELISA

Human CD25/IL-2 Rα Immunoassay

Catalog Number DR2A00
SR2A00
PDR2A00

For the quantitative determination of human Interleukin 2 Receptor alpha (IL-2 Rα) 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

The biological activities of IL-2 are mediated by its binding to a multi-molecular cellular receptor complex. For several years the receptor was thought to consist of two glycoprotein chains, an alpha chain (IL-2 Rα) and a beta chain (IL-2 Rβ) (1-3), which acted together to form a high affinity receptor that transduced the IL-2 signal. IL-2 Rα (also known as Tac antigen and as CD25) is a 55 kDa transmembrane glycoprotein composed of 351 amino acids with only 13 located on the cytoplasmic side of the membrane (4 - 6). The second chain of the complex was cloned in 1989 (7), and is a transmembrane glycoprotein of 575 amino acids (75 kDa), 286 of which are located cytoplasmically and clearly participate in signal transduction (8, 9). Eventually it was discovered that a third chain, IL-2 Rγ, was necessary for high affinity binding, ligand internalization and signalling. Constitutively expressed on many lymphoid cells, it had been overlooked partly because it has no affinity for IL-2 except when IL-2 Rβ is present (7, 10, 11). When cloned, the gene was found to code for a 64 kDa transmembrane protein of 347 amino acids, 84 of which are cytoplasmic (12). Both IL-2 Rβ and IL-2 Rγ are members of the hematopoietin receptor superfamily, whereas IL-2 Rα is related only to the IL-15 Rα chain (13-15).

A model of the IL-2 receptor complex (3, 9, 16-21) would describe the high affinity receptor as an αβγ trimer, in which all three chains are in contact with the ligand. Alone, IL-2 Rα binds IL-2 with low affinity, but is unable to transduce a signal. The αβ combination will bind IL-2 with intermediate affinity, but still will not transduce a signal. A βγ complex has intermediate affinity and is capable of signalling if the IL-2 concentration is relatively high. Regardless of many subtleties that determine the affinity of the ligand for the extracellular portions of the receptor components (22-24), signalling will ensue if the β and γ cytoplasmic domains are brought into close proximity (25-27).

A soluble form of IL-2 Rα appears in serum, concomitant with its increased expression on cells (18, 28, 29). There are reports of a soluble form of IL-2 Rβ as well (28, 30). The function of the soluble IL-2 Rα is unclear, since it would be expected to be a poor inhibitor of IL-2 because of its low binding affinity. In any case, increased levels of the soluble IL-2 Rα in biological fluids reportedly correlate with increased T and B cell activation and immune system activation. Results of a number of studies suggest a correlation of levels of IL-2 Rα in serum with the onset of rejection episodes in allograft recipients (18, 31-33), with activity of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis (SLE) (34) and with the course of some leukemias and lymphomas (35-40).

The Quantikine® Human CD25/IL-2 Rα Immunoassay is a 3.5 hour solid phase ELISA designed to measure soluble IL-2 Rα in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human IL-2 Rα and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-2 Rα showed linear 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 mass values for human IL-2 Rα.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-2 Rα has been pre-coated onto a microplate. Standards, samples, and conjugate are pipetted into the wells and any IL-2 Rα present is sandwiched by the immobilized antibody and the enzyme-linked polyclonal antibody specific for human IL-2 Rα. Following a wash to remove any unbound substances and/or antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-2 Rα bound. 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, further dilute the samples with calibrator diluent and repeat the assay. If cell culture supernate samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with calibrator diluent.
- 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 #	CATALOG # DR2A00	CATALOG # SR2A00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-2 Rα Microplate	890196	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-2 Rα.	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-2 Rα Standard	890198	2 vials	12 vials	Recombinant human IL-2 Rα in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human IL-2 Rα Conjugate	890197	1 vial	6 vials	11 mL/vial of a polyclonal antibody specific for human IL-2 Rα conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-1	895143	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6S	895142	2 vials	12 vials	21 mL/vial of animal serum with preservatives.	
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.

DR2A00 contains sufficient materials to run an ELISA on one 96 well plate.
SR2A00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDR2A00). 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.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human IL-2 Rα Controls (optional; R&D Systems®, Catalog # QC05).

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.*

SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate samples require at least a 10-fold dilution prior to the assay. A suggested 10-fold dilution is 25 µL of sample + 225 µL of Calibrator Diluent RD6S.

Serum and plasma samples require at least a 4-fold dilution prior to the assay. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD6S.

REAGENT PREPARATION

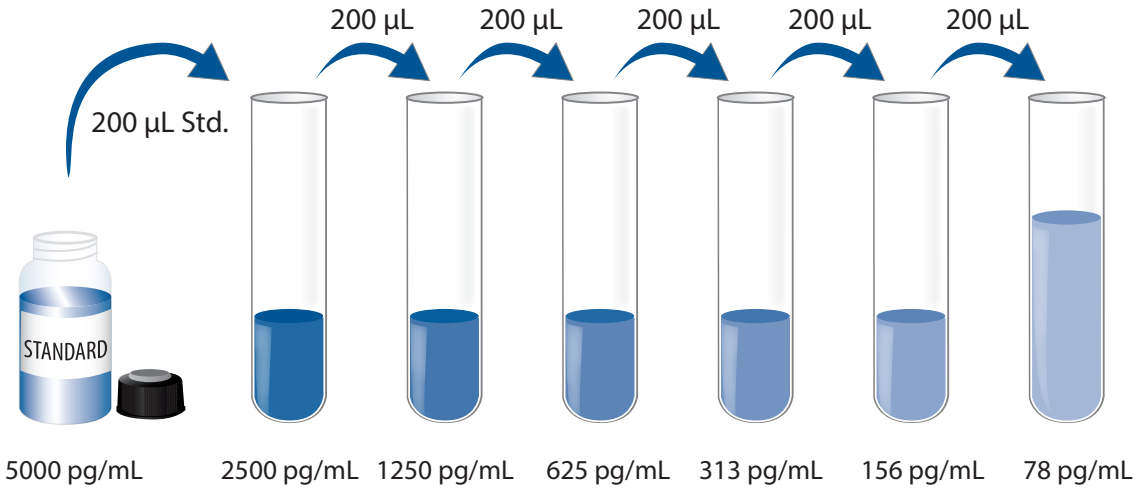
Bring all reagents to room temperature before use.

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-2 Rα Standard - **Refer to the vial label for standard reconstitution volume.** Reconstitute the Human IL-2 Rα Standard with Calibrator Diluent RD6S. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD6S 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-2 Rα Standard (5000 pg/mL) serves as the high standard. Calibrator Diluent RD6S 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, samples, 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. Add 100 µL of Assay Diluent RD1-1 to each well.
4. Add 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.

   Note: *Proceed to the next step. Do not incubate or wash.*
5. Add 100 µL of Human IL-2 Rα Conjugate to each well. Mix by gently tapping the plate on all four sides. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
6. 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.
7. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
8. 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.
9. 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

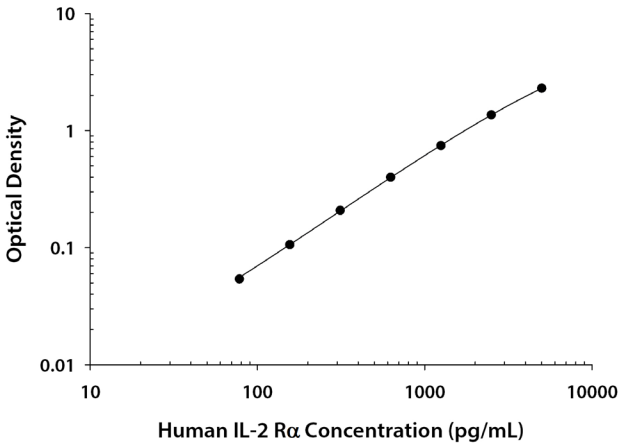
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human IL-2 Rα 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.

Since 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	0.014 0.014	0.014	—
78	0.068 0.067	0.068	0.054
156	0.119 0.122	0.120	0.106
313	0.227 0.218	0.222	0.208
625	0.407 0.419	0.413	0.399
1250	0.763 0.753	0.758	0.744
2500	1.337 1.415	1.376	1.362
5000	2.377 2.240	2.308	2.294

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	207	613	2357	188	630	2615
Standard deviation	12.7	37.5	108	13.6	38.1	183
CV (%)	6.1	6.1	4.6	7.2	6.0	7.0

RECOVERY

The recovery of human IL-2 Rα spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	105	93-118%
Serum (n=5)	105	99-116%
EDTA plasma (n=5)	103	91-113%
Heparin plasma (n=5)	104	98-113%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-2 Rα 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)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	98	98	97	101
	Range (%)	96-100	93-102	89-101	94-111
1:4	Average % of Expected	96	95	96	99
	Range (%)	89-101	90-99	88-100	95-105
1:8	Average % of Expected	95	94	90	98
	Range (%)	88-101	88-101	81-96	90-106
1:16	Average % of Expected	95	94	91	96
	Range (%)	89-100	91-99	86-92	84-104

SENSITIVITY

The minimum detectable dose (MDD) of human IL-2 Rα is typically less than 10 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 *Sf* 21-expressed recombinant human CD25/IL-2 Rα produced at R&D Systems®.

The NIBSC IL-2 Rα non-WHO reference material 97/600 was evaluated in this kit. The dose response curve of the reference material 97/600 parallels the Quantikine® kit standard curve. To convert sample values obtained with the Quantikine® Human CD25/IL-2 Rα kit to approximate NIBSC 97/600 units, use the equation below.

NIBSC (97/600) approximate value (IU/mL) = 0.0602 x Quantikine® IL-2 Rα value (pg/mL)

Note: *Based on data generated in October 2012.*

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-2 Rα in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=55)	1055	458-1997	365
EDTA plasma (n=55)	1062	410-2623	424
Heparin plasma (n=55)	1020	430-2082	365

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cell were cultured unstimulated or stimulated with 10 µg/mL of PHA for 1, 3, and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-2 Ra.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	245	459	645
Stimulated	3831	10,001	7765

SPECIFICITY

This assay recognizes natural and recombinant human IL-2 Rα.

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-2 Rα control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

gp130
IFN-γ
IL-1α
IL-1β
IL-1 RI
IL-1 RII
IL-1ra
IL-2
IL-2 Rβ
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-8
IL-9
IL-10
IL-11
IL-12
IL-13
M-CSF
TGF-β1
TGF-β3
TGF-β RII
TNF-α

Recombinant mouse:

IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
TNF-α

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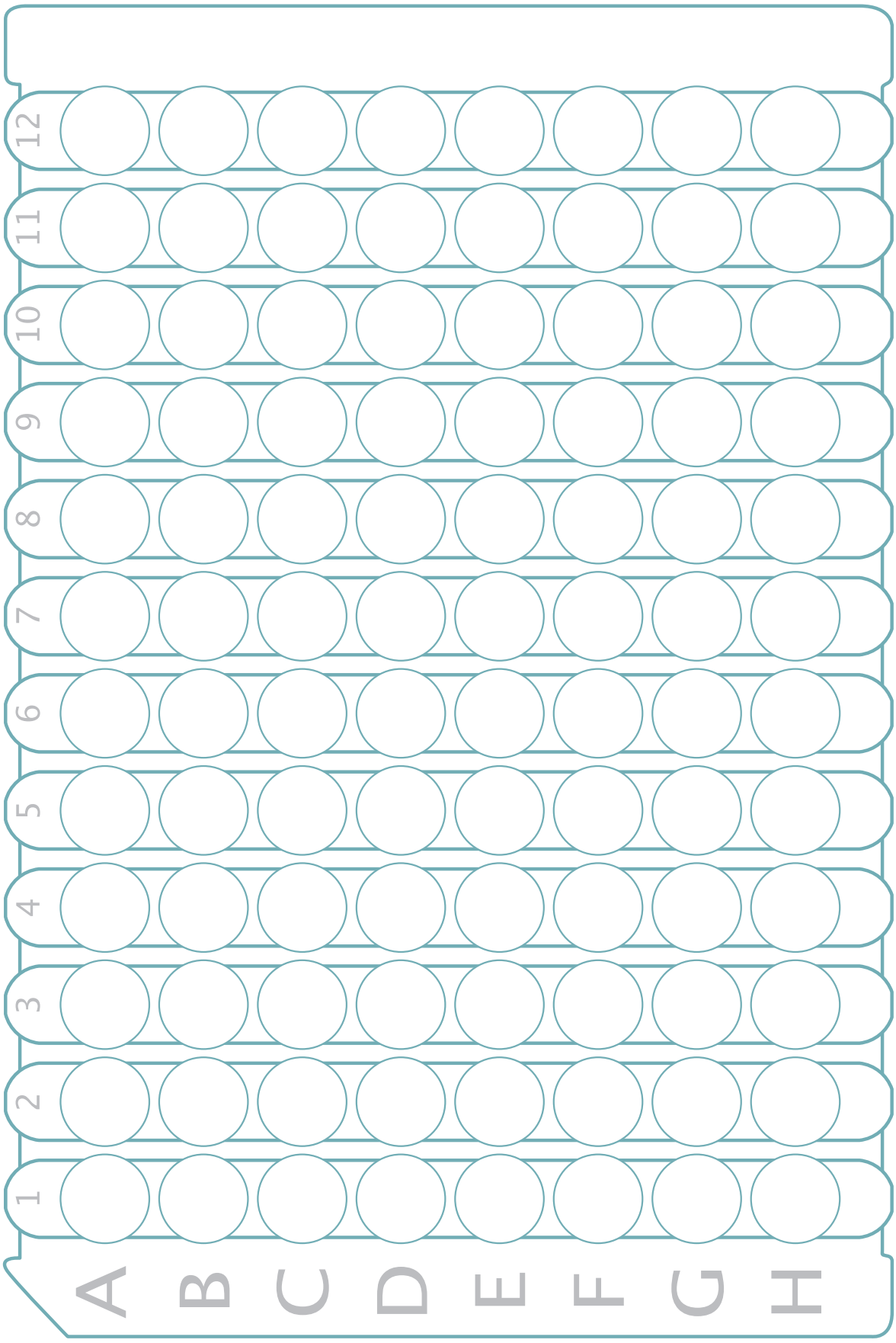
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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