Quantikine® ELISA

Human IL-33 Immunoassay

Catalog Number D3300

For the quantitative determination of human Interleukin 33 (IL-33) concentrations in cell culture supernates, cell lysates, 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.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	3
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
CELL LYSIS PROCEDURE	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
CALIBRATION	9
LINEARITY	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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INTRODUCTION

Interleukin-33 (IL-33), also known as NF-HEV and DVS 27, is a 30 kDa pro-inflammatory protein that plays an important role in Th2-biased immune responses and cardiac pathology (1-3). Human IL-33 is synthesized as a 270 amino acid (aa) molecule with an N-terminal nuclear localization signal, a helix-turn-helix motif, and a C-terminal region with structural homology to IL-1 family cytokines (4). Full length IL-33 interacts with nuclear chromatin, binds NFκB, and inhibits pro-inflammatory NFκB transactivation (5-7). Cleavage of full length IL-33 leads to the extracellular release of an 18-20 kDa C-terminal fragment known as mature IL-33 (4, 8). Cathepsin G, Elastase, and Proteinase 3 can each cleave full length IL-33, giving rise to N-terminal heterogeneity of the mature form (9, 10). IL-33 can be inactivated by further cleavage at several sites by Proteinase 3 and Caspase-1 (10, 11). Additional isoforms of human IL-33 with internal deletions are generated by alternative splicing (12, 13). Mature human IL-33 shares 57% and 59% aa sequence identity with mouse and rat IL-33, respectively.

IL-33 binds the transmembrane receptor ST2/IL-1 R4 which subsequently associates with IL-1 RAcP to enable IL-33 dependent signaling (4, 14-16). IL-1 RAcP is a shared signaling subunit that also associates with the receptors IL-1 RI, IL-1 RII, IL-1 R6, and SCF R/c-kit (17). A soluble isoform of ST2 retains the ability to bind IL-33 and blocks ST2-dependent responses (18, 19). Soluble IL-1 RAcP enhances the decoy function of soluble ST2 (16). IL-33 binding to transmembrane ST2 induces the association of ST2 with existing IL-1 RAcP/SCF R complexes (20). Activation of either ST2 or SCF R by their respective ligands can induce signal transduction through the other receptor subunit (20). IL-33 signaling through ST2 additionally triggers VE-Cadherin phosphorylation and internalization on vascular endothelial cells which leads to increased vascular permeability, vessel sprouting, and tubule formation (21).

IL-33 exerts multiple effects on immune system function. It acts on Th2 cells, basophils, and mast cells to induce their migration to sites of inflammation and production of Th2 cytokines (4, 22-26). IL-33 also promotes the expansion of regulatory T cells and alternately activated macrophages while attenuating Th17 cell expansion and activation (27). IL-33 contributes to infection clearance by enhancing neutrophil sensitization to TLR and Dectin-1 signaling, phagocytic activity, and migration to sites of infection (22, 28, 29). It is upregulated in a wide variety of cells under inflammatory conditions (5, 30, 31). Full length IL-33 is also found at elevated levels in bronchiolar lavage fluid during pulmonary fibrosis (32). The full length protein is classified as an alarmin due to its release from physically damaged or necrotic cells and its ability to trigger inflammatory and anti-viral $CD8^+$ T cell responses (11, 33). Like mature IL-33, the full length protein activates ST2 and promotes mast cell activation and neutrophil infiltration (8, 9, 11, 32).

IL-33 induces both protective and pathologic actions in the heart. It counteracts cardiac myocyte hypertrophy and responsiveness to angiotensin II and phenylephrine (18, 34). It is induced in cardiac fibroblasts by mechanical stress and circulates at elevated levels during chronic heart failure (as does the full length form) (18, 35). The soluble ST2 receptor is elevated in the serum of heart failure as well as asthma patients (18, 19). IL-33 inhibits the development of atherosclerotic plaques and induces the production of anti-oxidized LDL antibodies (23). It can also enhance eosinophilic perimyocarditis and impair heart function (34). In other settings, IL-33 limits neutrophil infiltration and circulating inflammatory chemokine levels following hepatic ischemia/reperfusion injury (36) but exacerbates $CD4^+$ T cell infiltration and tissue damage following cisplatin-induced acute kidney injury (37).

The Quantikine Human IL-33 Immunoassay is a 4.5 hour solid phase ELISA designed to measure IL-33 in cell culture supernates, cell lysates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-33 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-33. Results obtained using natural human IL-33 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-33.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for IL-33 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-33 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-33 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 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.
- 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
IL-33 Microplate	894424	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against IL-33.	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.*
IL-33 Conjugate	894425	21 mL of a polyclonal antibody against IL-33 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
IL-33 Standard	894426	4 ng of recombinant human IL-33 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-77	895545	17 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives.	
Cell Lysis Buffer 3	895366	21 mL of a concentrated buffered solution 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	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.
- 50 mL and 500 mL graduated cylinders.
- PBS (for cell lysates)
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-33 controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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. Please 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.*
Grossly lipemic or icteric samples are not recommended for use in this assay.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Perform a 5-fold dilution of Cell Lysis Buffer 3 with deionized or distilled water.
2. Wash cells three times in cold PBS.
3. Resuspend cells at 1 x 10^7 cells/mL of diluted Cell Lysis Buffer 3.
4. Incubate with gentle agitation for up to 30 minutes at room temperature and freeze/thaw cells once at ≤ -20 °C.
5. Centrifuge at 15,700 x g for 5 minutes to remove cell debris.
6. Assay immediately or aliquot the lysis supernates and store at ≤ -20 °C until ready for use.

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.

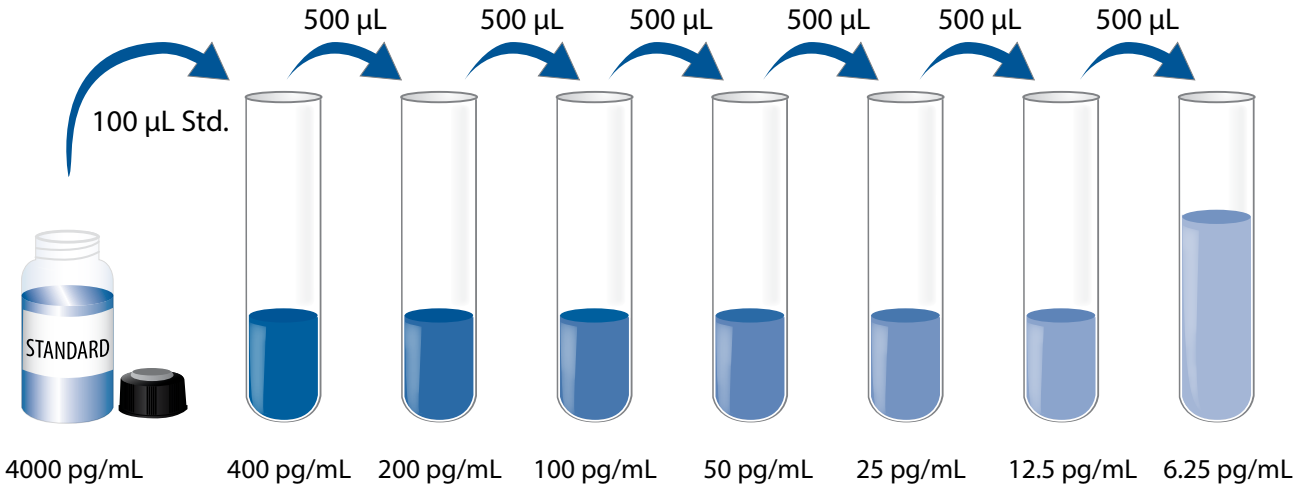
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (1:5) - *For serum/plasma samples.* Add 4 mL of Calibrator Diluent RD5-26 to 16 mL of deionized or distilled water to yield 20 mL of Calibrator Diluent RD5-26 (1:5).

Calibrator Diluent RD5-26 (1:20) - *For cell culture supernate/cell lysate samples.* Add 1 mL of Calibrator Diluent RD5-26 to 19 mL of deionized or distilled water to yield 20 mL of Calibrator Diluent RD5-26 (1:20).

IL-33 Standard - Reconstitute the IL-33 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-26 (1:5) (*for serum/plasma samples*) or Calibrator Diluent RD5-26 (1:20) (*for cell culture supernate/cell lysate samples*) into the 400 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 400 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 standards, samples, and controls 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 RD1-77 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm ± 50 rpm. 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 toweling.
6. Add 200 µL of IL-33 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 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.).

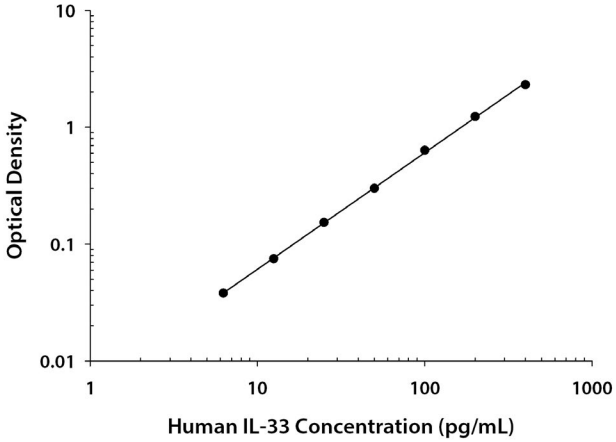
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the IL-33 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

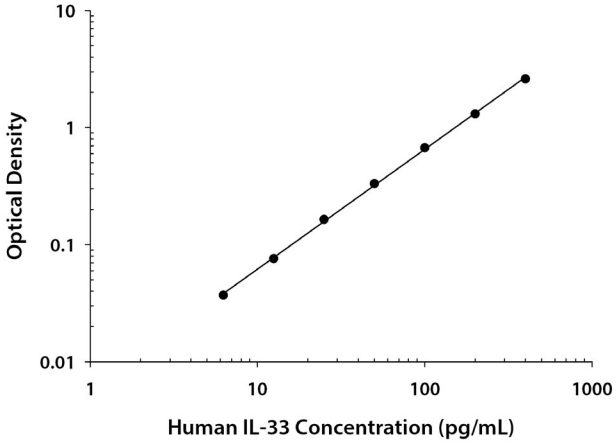
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD5-26 (1:5)



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.038	0.037	—
6.25	0.073 0.077	0.075	0.038
12.5	0.111 0.112	0.112	0.075
25	0.190 0.190	0.190	0.153
50	0.335 0.339	0.337	0.300
100	0.670 0.674	0.672	0.635
200	1.246 1.295	1.271	1.234
400	2.321 2.374	2.348	2.311

CALIBRATOR DILUENT RD5-26 (1:20)



(pg/mL)	O.D.	Average	Corrected
0	0.040 0.040	0.040	—
6.25	0.076 0.078	0.077	0.037
12.5	0.115 0.117	0.116	0.076
25	0.200 0.207	0.204	0.164
50	0.363 0.381	0.372	0.332
100	0.700 0.721	0.711	0.671
200	1.292 1.404	1.348	1.308
400	2.561 2.722	2.642	2.602

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 over two lots of kit components. Assays were performed by at least three technicians.

CELL CULTURE SUPERNATE/CELL LYSATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	34.1	112	214	36.4	116	224
Standard deviation	1.21	3.22	5.36	2.8	6.3	10.4
CV (%)	3.5	2.9	2.5	7.7	5.4	4.6

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)	34.3	111	218	39.0	120	230
Standard deviation	1.45	3.33	5.00	2.2	5.6	12.8
CV (%)	4.2	3.0	2.3	5.6	4.7	5.6

RECOVERY

The recovery of IL-33 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	95	81-106%
Serum (n=4)	96	88-103%
EDTA plasma (n=4)	86	82-92%
Heparin plasma (n=4)	84	80-90%

SENSITIVITY

Fifty-one assays were evaluated and the minimum detectable dose (MDD) of IL-33 ranged from 0.187-1.65 pg/mL. The mean MDD was 0.519 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density 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-33 produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of IL-33 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Cell lysates* (n=3)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	98	100	99	104
	Range (%)	96-103	92-107	98-102	93-107	100-109
1:4	Average % of Expected	95	96	101	105	109
	Range (%)	92-100	89-104	96-105	97-113	104-114
1:8	Average % of Expected	94	93	95	101	102
	Range (%)	89-100	88-99	91-101	92-111	92-111
1:16	Average % of Expected	94	88	103	106	115
	Range (%)	92-97	83-94	97-109	98-116	106-120

*Sample were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of IL-33 in this assay. No medical histories were available for the donors used in this study. No detectable levels were observed.

Cell Culture Supernates/Cell Lysates:
THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum and 50 µM β-mercaptoethanol until confluent. Cells were cultured unstimulated or stimulated with 5 µg/mL LPS and 50 nM PMA for 24 hours.

Sample Type	Stimulated (pg/mL)	Unstimulated
Cell culture supernate	49.6*	ND
Cell lysate	142	ND

**After 24 hours of stimulation, some cells were dead and lysed. This may have resulted in intracellular IL-33 contributing to the level of IL-33 observed in the cell culture supernate sample.*

SPECIFICITY

This assay recognizes natural and recombinant human IL-33.

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 IL-33 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1α
IL-1β
IL-1β (pro)
IL-1F7
IL-1Ra
IL-1 RAcP
IL-1 RAPL1
IL-1 RAPL2
IL-1 Rrp2
IL-18
IL-18 Rα
IL-18 Rβ
IL-36α
IL-36β
IL-36γ
IL-36Ra
IL-37
IL-38
ST2/IL-1 R4

Recombinant mouse:

IL-1β
IL-1 RAPL2
IL-36α
IL-36β
IL-36γ
IL-36Ra
IL-18
ST2

Recombinant rat:

IL-1α
IL-1β
IL-1 Ra
IL-18

Recombinant equine:

IL-1β
IL-1Ra

Recombinant porcine:

IL-1α
IL-1β
IL-1Ra
IL-18

Recombinant cotton rat:

IL-1α
IL-1β

Recombinant rhesus macaque:

IL-1β
IL-18

Other recombinants:

canine IL-1β
guinea pig IL-1β
feline IL-18

Recombinant mouse IL-33 cross-reacts approximately 0.21% in this assay.

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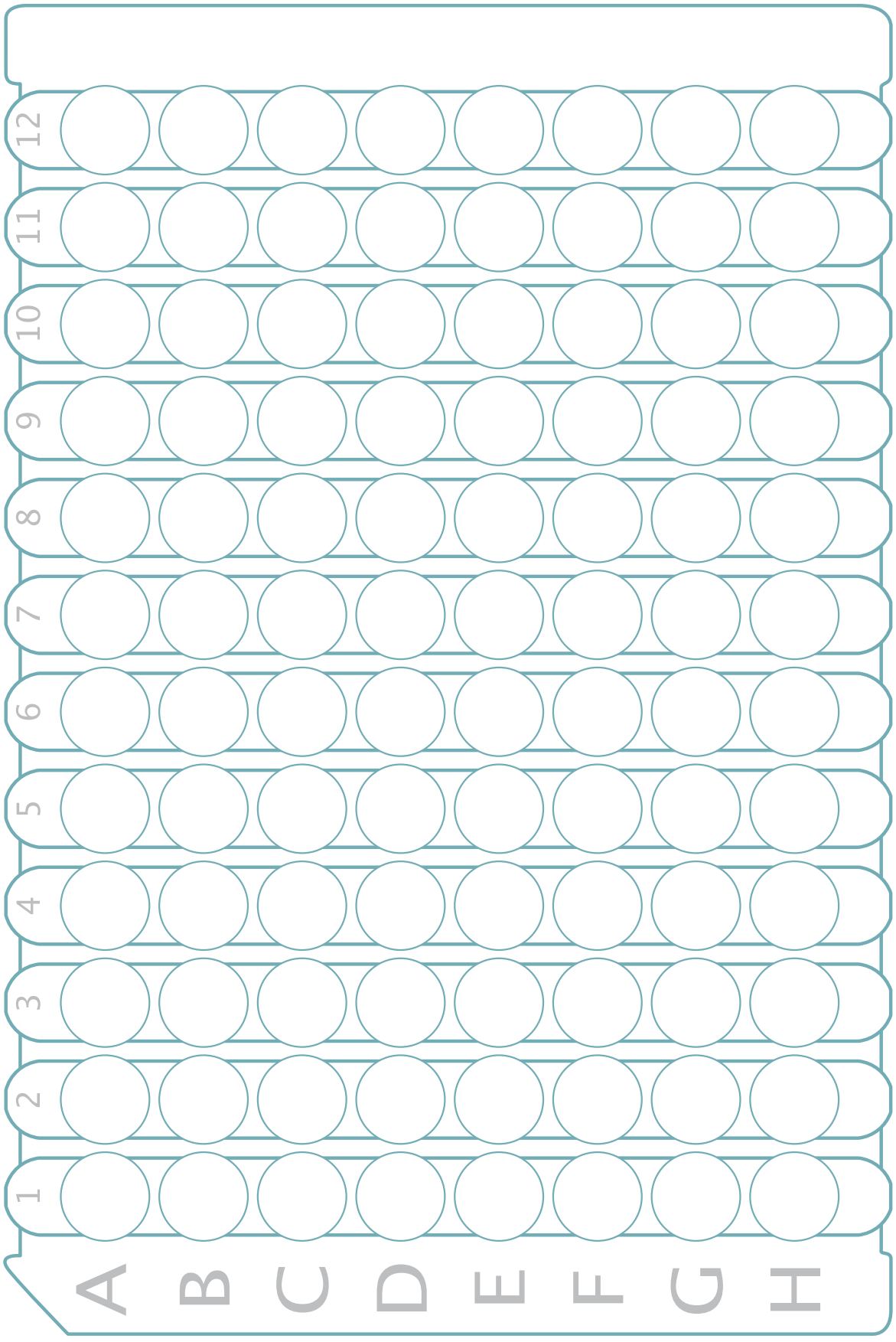
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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