Quantikine®

Porcine IL-1β/IL-1F2 Immunoassay

Catalog Number PLB00

For the quantitative determination of porcine interleukin 1 beta (IL-1β) concentrations in cell culture supernates.

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

IL-1β possesses a wide range of biological effects and plays a central role in immune and inflammatory response, bone remodeling, fever, carbohydrate metabolism and GH/IGF-I physiology. The production of IL-1β is tightly regulated. IL-1β is induced by microbial products and other factors related to injury or infection. The secretion of IL-1β is essential for initiating a cascade of events that are aimed at reestablishing homeostasis. Prolonged or inappropriate secretion of IL-1β, however, is associated directly or indirectly with a number of pathological conditions including leukemia, atherosclerosis, rheumatoid arthritis/autoimmune disease, and diabetes mellitus (1 - 3).

Porcine interleukin 1 beta (IL-1β) is an 18 kDa secreted protein that is involved in a number of inflammatory conditions (4). It is synthesized as a 267 amino acid (aa), 31 kDa proprotein, and is proteolytically cleaved by porcine IL-1β converting enzyme (ICE) to form a 114 aa prosegment and a 153 aa mature segment that is secreted (4 - 6). Pro-IL-1β lacks a signal sequence and this has led to the suggestion that nonclassical pathways are involved in the secretion of IL-1β (7). Although pro-IL-1β has no known biological functions, the pro-peptide has been shown to interact with the mature domain, suggesting that the pro-peptide may modulate the biological activity of mature IL-1β (8). Mature porcine IL-1β shows 69% and 67% aa identity to human and mouse IL-1β, respectively (5). Porcine IL-1β also shares 24% aa identity with porcine IL-1α. This is consistent with the 25% homology observed between human IL-1β and IL-1α (9, 10). Porcine cells known to express IL-1β include macrophages (11, 12), fibroblasts (13), and vascular endothelium (13, 14). Porcine IL-1β is reportedly active on bovine endothelial cells (5).

The signaling receptor complex for IL-1β consists of at least two components, an 80 kDa ligand-binding subunit (IL-1 RI) that participates in signal-transduction, and a 66 kDa non-ligand binding subunit (IL-1 RacP) that interacts with IL-1 RI. In addition, there is a non-signaling, ligand-binding subunit (IL-1 RII) that is considered a decoy receptor and acts alone (15 - 18). It likely serves to limit the availability to bioactive IL-1β (1). In humans, IL-1 RI and IL-1 RII show less than 30% aa homology with each other (15, 16). The porcine receptor subunits have not yet been cloned.

The Quantikine Porcine IL-1β Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-1β in cell culture supernates. It contains *E. coli*-expressed recombinant mature porcine IL-1β and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant porcine IL-1β. Results obtained using natural porcine IL-1β show dose response curves that are parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Porcine IL-1β Immunoassay kit can be used to determine relative mass values for natural porcine IL-1β.

Pro-IL-1β has not been tested in this immunoassay kit. It is possible that this kit may cross-react with and underestimate porcine pro-IL-1β in samples. However, in biological samples other than cell lysates, the precursor form of IL-1β (which is not bioactive) is usually not the predominant form of IL-1β. Therefore, results obtained using the Quantikine Porcine IL-1β Immunoassay kit should provide a useful measure of the levels of bioactive porcine IL-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for porcine IL-1β has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any porcine IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for porcine IL-1β 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 porcine IL-1β 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, dilute the samples with the appropriate 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 the appropriate Calibrator Diluent.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the receptors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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

Porcine IL-1β Microplate (Part 890862) - One 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody specific for porcine IL-1β.

Porcine IL-1β Conjugate (Part 890864) - 21 mL of a polyclonal antibody against porcine IL-1β conjugated to horseradish peroxidase with preservatives.

Porcine IL-1β Standard (Part 890863) - 3 vials (5 ng/vial) of recombinant porcine IL-1β in a buffered protein base with preservatives; lyophilized.

Porcine IL-1β Control (Part 890145) - 3 vials of recombinant porcine IL-1β in a buffered protein base with preservatives; lyophilized. The concentration range of porcine IL-1β after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.

Assay Diluent RD1-63 (Part 895352) - 12.5 mL of a buffered protein solution with preservatives.

Calibrator Diluent RD5-27 (Part 895395) - 21 mL of a buffered protein solution with preservatives.

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid solution.

Plate Covers (Part 640197) - 4 adhesive plate sealers.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use beyond kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Porcine IL-1β Conjugate</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Diluted Wash Buffer</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD5-27</td></tr>
<tr><td>Assay Diluent RD1-63</td></tr>
<tr><td>Color Reagent A</td></tr>
<tr><td>Color Reagent B</td></tr>
<tr><td>Porcine IL-1β Standard</td><td rowspan="2">Use a new Standard and Control for each assay.</td></tr>
<tr><td>Porcine IL-1β Control</td></tr>
<tr><td>Microplate Wells</td><td>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.*</td></tr>
</table>

*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.
- **Polypropylene** tubes for serial dilution.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

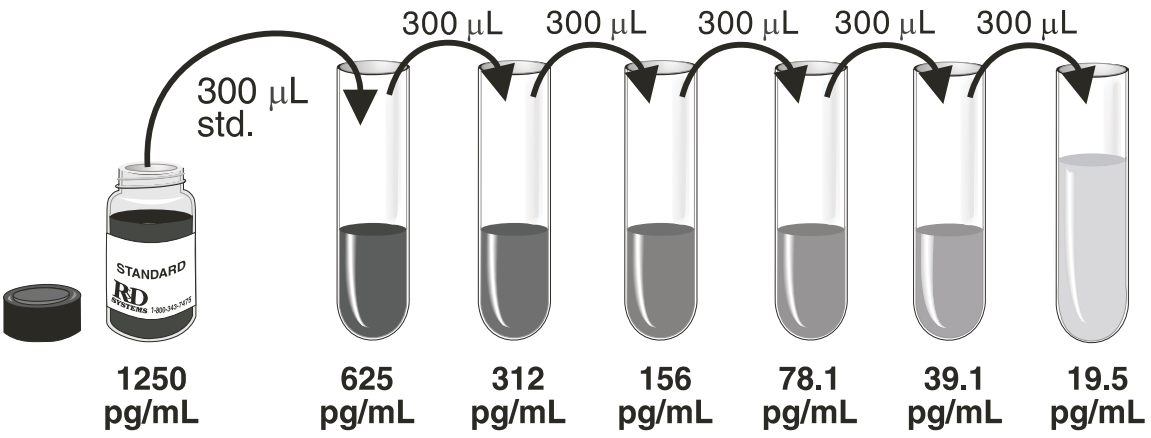
Porcine IL-1β Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 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. 120 µL of the resultant mixture is required per well.

Porcine IL-1β Standard - Reconstitute the porcine IL-1β Standard with 4.0 mL of Calibrator Diluent RD5-27. Do not substitute other diluents. This reconstitution produces a stock solution of 1250 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 300 µL of Calibrator Diluent RD5-27 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted porcine IL-1β Standard serves as the high standard (1250 pg/mL). Calibrator Diluent RD5-27 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, standards, and control be assayed in duplicate.

1. Prepare reagents, samples, and standard dilutions as directed by 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 RD1-63 to each well.

4. Add 100 µL of Standard, Control, or sample per well. Mix by gently tapping the plate frame for 1 minute. 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 four times for a total of five 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 porcine IL-1β 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 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

9. Add 120 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.

PROCEDURE SUMMARY AND CHECKLIST

1. ☐ Bring all reagents to room temperature.
☐ Prepare reagents and samples as instructed.
☐ Return unused components to storage temperature as indicated in the instructions.

2. ☐ Add 50 µL Assay Diluent to each well.

3. ☐ Add 100 µL Standard, Control, or sample to each well.
☐ Tap plate gently for one minute.
☐ Cover the plate and incubate for 2 hours at room temperature.

4. ☐ Aspirate and wash each well five times.

5. ☐ Add 200 µL Conjugate to each well.
☐ Cover the plate and incubate for 2 hours at room temperature.

6. ☐ Aspirate and wash each well five times.

7. ☐ Add 120 µL Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

8. ☐ Add 120 µL Stop Solution to each well.

9. ☐ Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

CALCULATION OF RESULTS

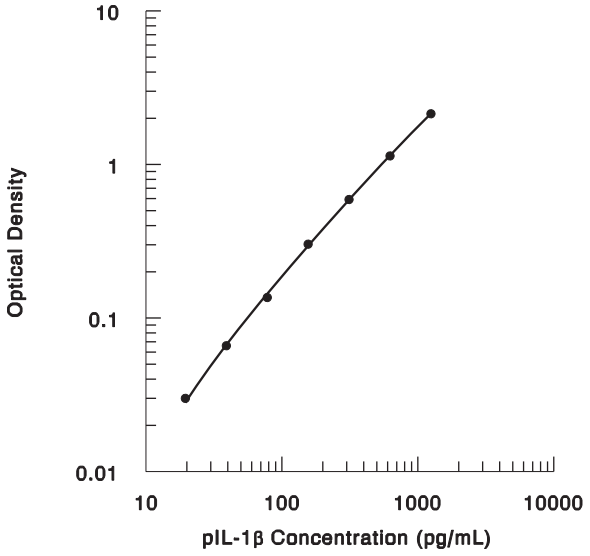
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 porcine IL-1β 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 prior to assay, the concentration read from the standard curve must be multiplied by the respective 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	0.081 0.084	0.082	—
19.5	0.105 0.119	0.112	0.030
39.1	0.144 0.153	0.148	0.066
78.1	0.216 0.220	0.218	0.136
156	0.364 0.406	0.385	0.303
312	0.657 0.690	0.674	0.592
625	1.198 1.241	1.220	1.138
1250	2.165 2.280	2.222	2.140

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.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	108	283	1095	106	239	1171
Standard deviation	9.2	12.6	35.8	11.0	22.0	108
CV (%)	8.5	4.4	3.3	10.4	9.2	9.2

SENSITIVITY

The minimum detectable dose of porcine IL-1β is typically less than 10 pg/mL.

The minimum detectable dose is determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

RECOVERY

The recovery of porcine IL-1β spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	95	86 - 107%

LINEARITY

To assess the linearity of the assay, five samples containing and/or spiked with various concentrations of porcine IL-1β were diluted with Calibrator Diluent and assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell Culture Supernates	neat	652		
	1/2	322	326	99
	1/4	161	163	99
	1/8	78	82	95
	1/16	40	41	98

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant porcine IL-1β produced at R&D Systems. The recombinant form of porcine IL-1β contains 153 amino acid residues including the amino terminal methionyl group and has a predicted molecular mass of 18 kDa.

Based on total amino acid analysis, the absorbance of a 1 mg/mL solution of the *E. coli*-expressed recombinant porcine IL-1β at 280 nm was determined to be 0.91 A.U.

SAMPLE VALUES

Porcine PBL cells (5 x 10^6 cells/mL) were cultured for 6 days in DMEM plus 10% fetal bovine serum, and stimulated twice with 100 ng/mL LPS at day 0 and day 4. The cell culture supernate was assayed for porcine IL-1β and measured 3.8 ng/mL.

Porcine PBL cells (5 x 10^6 cells/mL) were stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin for 4 days. The cell culture supernate was assayed for porcine IL-1β and measured 2.1 ng/mL.

SPECIFICITY

This assay recognizes both recombinant and natural porcine IL-1β. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range porcine IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:
IL-1α
IL-4
IL-6
IL-8
IL-10
PDGF
TGF-β
TNF-α

Some cross-reactivity was observed with the following:

Recombinant Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
mouse IL-1β	50,000	488	1.0
rat IL-1β	50,000	1835	3.7
human IL-1β	50,000	107	0.2

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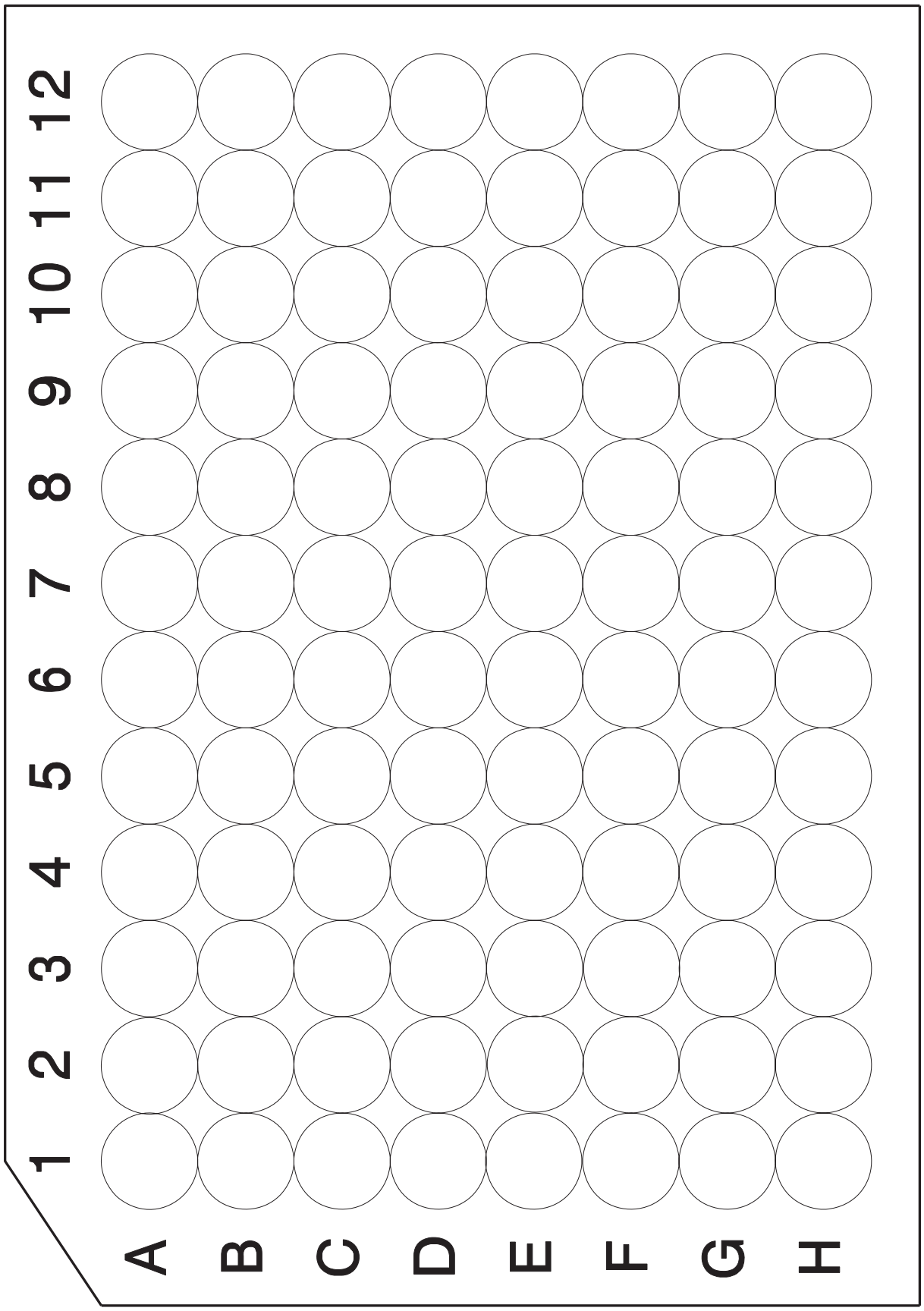
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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