Quantikine®

Mouse IL-20 Immunoassay

Catalog Number ML200

For the quantitative determination of mouse interleukin 20 (IL-20) concentrations in cell culture supernates, mouse 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

Interleukin 20 (IL-20; also known as IL-10F2) was identified in an EST database search for α-helical cytokines (1 - 3). It is an 18 kDa, monomeric, non-glycosylated polypeptide that belongs to the IL-19 subfamily of the IL-10 family of proteins (2 - 5). IL-20 is synthesized as a 176 amino acid (aa) precursor that contains a 24 aa signal sequence and a 152 aa mature region (1). The mature region contains three intrachain disulfide bonds and no potential N-linked glycosylation sites. Relative to IL-10, which has only two intrachain disulfide bonds and forms a dimer, the existence of the third disulfide bond in IL-20 precludes the formation of a covalent dimer (2). Cells known to express IL-20 include monocytes (6), breast and colon epithelial cell lines (6), and keratinocytes (2, 5, 7). Mature mouse IL-20 shows 77%, 76%, and 70% aa sequence identity to human (1), canine (8) and bovine (9) IL-20, respectively. Human IL-20 is active on mouse cells (1).

IL-20 signals through binding to two types of high affinity IL-20 receptor complexes: IL-20 R1 (also known as IL-20 RA/IL-20 Rα/CRF2-8) and IL-20 R2 (also known as IL-20 RB/ IL-20 Rβ/CRF2-11) or IL-22 R (also known as IL-22 R1/IL-22 RA/CRF2-9) and IL-20 R2 (4, 10 - 15). All receptor subunits are type I transmembrane proteins that belong to the class II cytokine receptor family. Binding to either receptor complexes induces STAT3 activation over STAT1 (1, 4, 14). In addition to IL-20, multiple IL-10 family members including IL-19, IL-24, and Yaba-like disease virus also utilize the two high affinity IL-20 receptor complexes (2, 14, 16).

IL-20 has been most associated with keratinocyte dysfunction (1, 5 - 7, 17). Over-expression of IL-20 in neonatal mice leads to a psoriasis-like condition with thickened epidermis, hyperkeratosis, and compact stratum corneum. IL-20 has been shown to induce KGF production from $CD8^+$ T cells, which indirectly induce keratinocyte proliferation (5). IL-20 has other effects, including the induction of TNF-α secretion in keratinocytes and monocytes (7), the downregulation of COX-2/PGE_2 in bronchial epithelium (18), and the promotion of CFU-GEMM production by multipotent hematopoietic progenitor cells (19).

The Quantikine Mouse IL-20 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-20 in cell culture supernates, mouse serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-20 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-20 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Mouse IL-20 kit can be used to determine relative mass values for naturally occurring mouse IL-20.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-20 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse IL-20 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse 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. 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 mouse IL-20 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 Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested, 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.

REAGENTS

Mouse IL-20 Microplate (Part 892954) - One 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-20.

Mouse IL-20 Conjugate (Part 892955) - 12 mL of a polyclonal antibody against mouse IL-20 conjugated to horseradish peroxidase with preservatives.

Mouse IL-20 Standard (Part 892956) - 1 vial of recombinant mouse IL-20 in a buffered protein base, with preservatives, lyophilized.

Mouse IL-20 Control (Part 892957) - 1 vial of recombinant mouse IL-20 in a buffered protein base with preservatives, lyophilized. The concentration range of IL-20 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 RD1W (Part 895038) - 12 mL of a buffered protein solution with preservatives.

Calibrator Diluent RD6-12 (Part 895214) - 21 mL of diluted animal serum with preservatives.

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

Color Reagent A (Part 895000) - 12 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12 mL of stabilized chromogen (tetramethylbenzidine).

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

Plate Covers (Part 640197) - 4 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Mouse IL-20 Conjugate</td><td rowspan="9">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>Assay Diluent RD1W</td></tr>
<tr><td>Calibrator Diluent RD6-12</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Mouse IL-20 Standard (1000 pg/mL)</td></tr>
<tr><td>Mouse IL-20 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.

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.

Serum - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at approximately 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: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay. Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

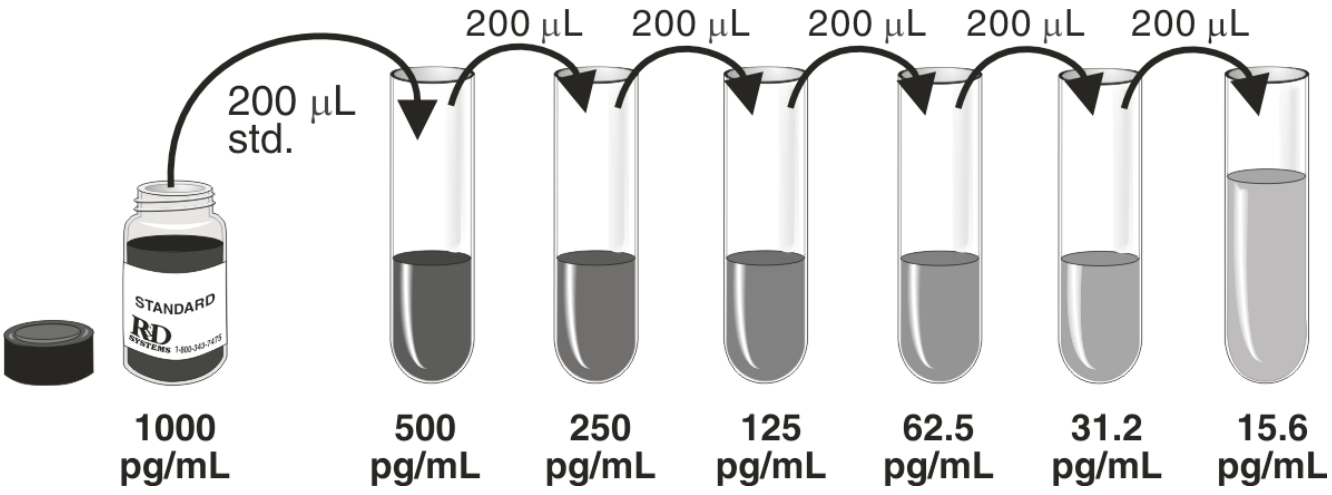
Mouse IL-20 Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

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 of 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. 100 µL of the resultant mixture is required per well.

Mouse IL-20 Standard - Reconstitute the mouse IL-20 Standard with 5.0 mL of Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-12 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, control, and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, 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, reseal.

3. Add 50 µL of Assay Diluent RD1W to each well.

4. Add 50 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 100 µL of mouse 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **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.

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 50 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 100 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 100 µL Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

8. ☐ Add 100 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

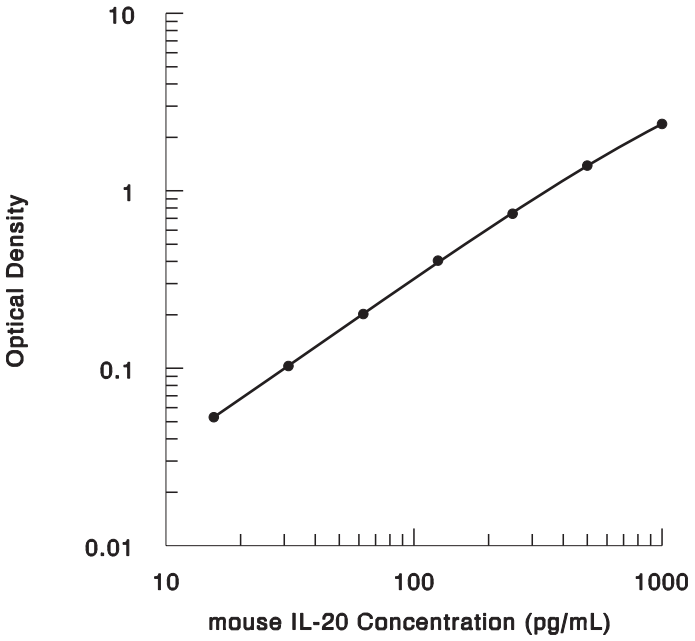
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 mouse IL-20 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 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 0.021	0.019	—
15.6	0.071 0.072	0.072	0.053
31.2	0.120 0.123	0.122	0.103
62.5	0.217 0.224	0.221	0.202
125	0.407 0.438	0.423	0.404
250	0.739 0.785	0.762	0.743
500	1.333 1.478	1.406	1.387
1000	2.325 2.473	2.399	2.380

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 at least thirty-six separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	36	38	36
Mean (pg/mL)	36.3	116	414	37.6	117	431
Standard deviation	3.6	7.1	22.6	4.6	8.9	42.0
CV (%)	9.9	6.1	5.5	12.2	7.6	9.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Mouse cell culture supernates (n=4)	102	91 - 113%
Mouse serum (n=4)	105	92 - 114%
Mouse heparin plasma (n=4)	95	88 - 107%
Mouse EDTA plasma (n=4)	98	91 - 111%

LINEARITY

To assess the linearity of the assay, samples containing or spiked with high concentrations of mouse IL-20 in each matrix were diluted with Calibrator Diluent RD6-12 and then assayed.

		Cell culture supernates (n=5)	Serum (n=4)	Heparin plasma (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	94	98	96	105
	Range (%)	91 - 97	95 - 102	91 - 101	97 - 108
1:4	Average % of Expected	91	102	95	99
	Range (%)	85 - 98	96 - 105	88 - 105	87 - 106
1:8	Average % of Expected	89	107	97	99
	Range (%)	84 - 93	102 - 110	89 - 110	87 - 109
1:16	Average % of Expected	93	106	101	105
	Range (%)	85 - 100	96 - 112	90 - 118	99 - 115

SENSITIVITY

Thirty-three assays were evaluated and the minimum detectable dose (MDD) of mouse IL-20 ranged from 1.3 - 6.4 pg/mL. The mean MDD was 3.0 pg/mL.

The minimum detectable dose 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 mouse IL-20 produced at R&D Systems.

The protein concentration of the recombinant mouse IL-20 was determined by the method of Bradford (20) using purified bovine serum albumin as a standard.

SAMPLE VALUES

Serum/Plasma - Individual mouse serum and plasma samples were evaluated for detectable levels of mouse IL-20 in this assay. Serum and plasma samples are not matched.

Mouse Samples	Mean (pg/mL)	Std. Dev. (pg/mL)	Range (pg/mL)	% Detectable
Mouse serum (n=17)	49	27	17 - 121	100
Mouse heparin plasma (n=10)	81	—	ND - 128	70
Mouse EDTA plasma (n=10)	70	38	20 - 128	100

ND = Non-detectable

SPECIFICITY

This assay recognizes both recombinant and natural mouse IL-20. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6-12 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse IL-20 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
IL-1α
IL-1β
IL-1 R4
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 sR
IL-11
IL-11 Rα/Fc Chimera
IL-12
IL-12 p40
IL-13
IL-13 Rα1/Fc Chimera
IL-15
IL-17
IL-18
IL-19
IL-20 Rα/Fc Chimera
IL-21
IL-22
IL-22 BP/His
IL-24
IL-28/IFN-λ

Recombinant rat:
IL-22

Recombinant human:
IL-20
IL-22
IL-22 R/Fc Chimera

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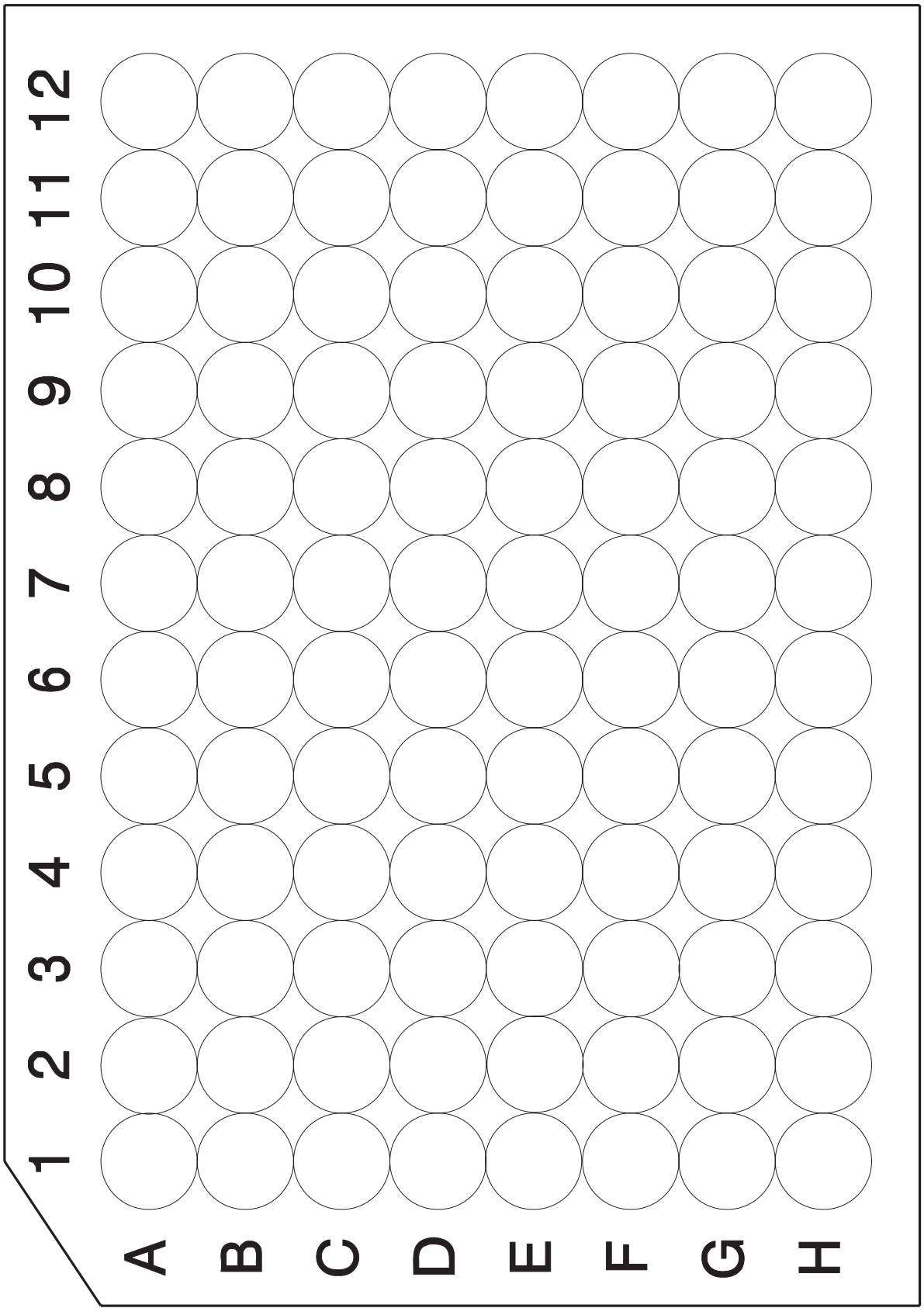
PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



NOTES