# QuantiGlo® ELISA

## Human IL-4 Immunoassay

Catalog Number Q4000

For the quantitative determination of human Interleukin 4 (IL-4) 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

Interleukin 4 (IL-4) is a pleiotropic cytokine produced primarily by activated T lymphocytes, mast cells and basophils (1-3). IL-4 has multiple immune response-modulating functions on a variety of cell types. It is an important regulator of isotype switching, inducing IgE production in B lymphocytes. It is an important modulator of the differentiation of precursor T helper cells to the Th2 subset that mediates humoral immunity and modulates antibody production. In addition, IL-4 has also been shown to have anti-tumor activity both *in vivo* and *in vitro* (1-3).

The sequence of human IL-4 cDNA predicts a 153 amino acid (aa) residue precursor protein containing a 24 aa residue signal peptide that is cleaved to form the mature protein (4). At the amino acid sequence level, mature human IL-4 is approximately 50% identical to mouse IL-4 and there is no species cross-reactivity between the two proteins (1, 2). Human IL-4 also shares approximately 30% amino acid sequence identity to human IL-13 and the two cytokines exhibit overlapping biological activities (5, 6). The gene for IL-4 has been mapped to human chromosome 5q, in close proximity to the genes for IL-3, IL-5, IL-13, and GM-CSF (1, 2).

The biological effects of IL-4 are mediated by specific cell surface receptor complexes. One type of functional IL-4 receptor complex consists of the IL-4-binding subunit (IL-4 R) and a second chain, designated the common γ chain because it has also been identified as a component of the receptor complexes for IL-2, IL-7, IL-9, and IL-15 (7 - 9). A second type of functional IL-4 receptor complex, consisting of the IL-4 R and IL-13 Rα, has also been proposed (10, 11). Although IL-4 R does not bind IL-13 directly, it has been shown to complex with the low-affinity IL-13 Rα to form the functional high-affinity receptor complex for IL-13 (11, 12). In addition to the membrane-bound form of IL-4 R, a naturally occurring soluble form of IL-4 R has been identified in human and mouse biological fluids and in mouse cell culture supernates (13-15). Soluble IL-4 R has been to shown to bind IL-4 with high affinity in solution.

The QuantiGlo Human IL-4 Chemiluminescent Immunoassay is a 5.5 hour solid phase ELISA designed to measure human IL-4 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-4 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-4. Results obtained using natural human IL-4 showed dose response curves that were parallel to the standard curves obtained using the recombinant QuantiGlo kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-4.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-4 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution (16) is added to the wells and light is produced in proportion to the amount of IL-4 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

## TECHNICAL HINTS

- 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.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the QuantiGlo Immunoassay, the possibility of interference cannot be excluded.
- Variation in pipetting technique, washing technique, luminometers, incubation time or temperature can cause variations in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- 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.
- Relative light units (RLUs) may differ among luminometers. Adjust settings as recommended by the instrument manufacturer.
- Relative light units may vary within the 20 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low beginning with the high standard in wells $A_1$ and $A_2$.

## 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-4 Microplate** | 890620 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-4. | 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-4 Conjugate** | 890621 | 21 mL of a polyclonal antibody against IL-4 conjugated to horseradish peroxidase with preservative. | May be stored for up to 1 month at 2-8 °C.* |
| **IL-4 Standard** | 890622 | 12.5 ng of recombinant human IL-4 in a buffered protein base with preservative; lyophilized. | |
| **Assay Diluent RD1-35** | 895271 | 11 mL of a buffered protein base with blue dye and preservative. | |
| **Calibrator Diluent RD5P Concentrate** | 895151 | 21 mL of buffered protein base with preservatives. *For cell culture supernate samples.* | |
| **Calibrator Diluent RD6N** | 895135 | 21 mL of animal serum with preservatives. *For serum/plasma samples.* | |
| **Wash Buffer Concentrate** | 895222 | 100 mL of a 10-fold concentrated solution of buffered surfactant with preservative. | |
| **Substrate A** | 895224 | 12 mL of stabilized enhanced luminol. | |
| **Substrate B** | 895225 | 12 mL of stabilized hydrogen peroxide. | |
| **Plate Sealers** | N/A | 4 adhesive strips. | |

* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Luminometer set with the following parameters: 1.0 minute lag time; 0.5 sec/well read time; summation mode; auto gain on, or equivalent.
- Pipettes and pipette tips.
- 100 mL and 1 liter graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-4 Controls (optional; available from R&D Systems).

## 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, heparin, or citrate 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:** *Hemolyzed samples are not suitable for use in this assay.*

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

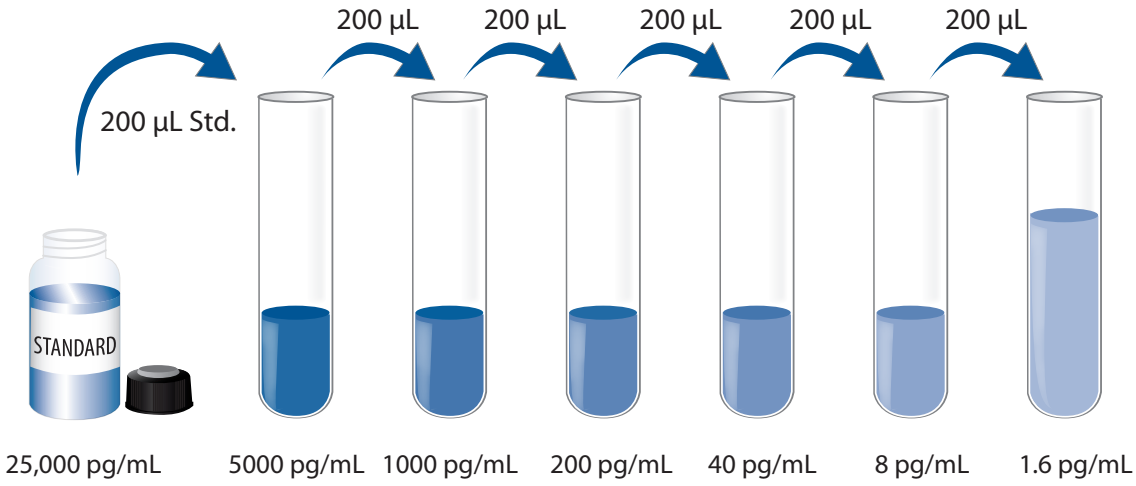
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

**Substrate Solution** - Substrates A and B should be mixed together in equal volumes 15 minutes to 4 hours before use. Store in a capped plastic container, protected from light. 200 µL of the resultant mixture is required per well.

**Calibrator Diluent RD5P (1X)** - Add 20 mL of Calibrator Diluent RD5P Concentrate to 20 mL of deionized or distilled water to yield 40 mL of Calibrator Diluent RD5P (1X).

**IL-4 Standard** - Reconstitute Standard with 0.5 mL of deionized or distilled water. This reconstitution produces a stock solution of 25,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 800 µL of Calibrator Diluent RD5P (1X) (*for cell culture supernate samples*) or Calibrator Diluent RD6N (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5000 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 RD1-35 to each well.
4. Add 150 µL of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 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 towels.
6. Add 200 µL of IL-4 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.

   **Note:** *Prepare Substrate Solution at this time.*
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20-40 minutes at room temperature **on the benchtop (do not shake).**
9. Determine the RLU of each well using a luminometer set with the following parameters: 1.0 min. lag time; 1 sec/well read time; summation mode; auto gain on, or equivelant.

## CALCULATION OF RESULTS

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

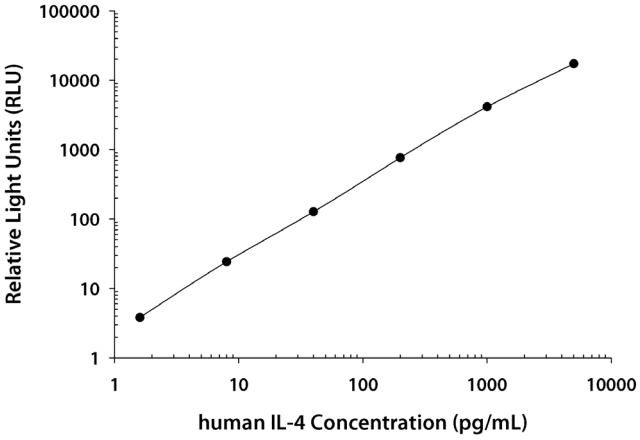
Create a standard curve by reducing the data using computer software capable of generating a cubic-spline curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

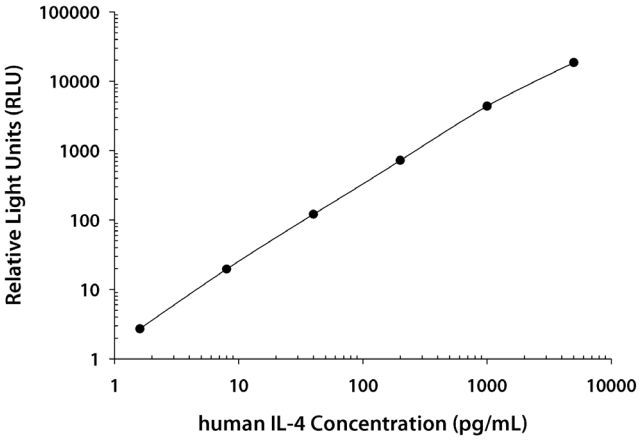
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CALIBRATOR DILUENT RD5P (1X)




| (pg/mL) | RLU | Average | Corrected |
|---|---|---|---|
| 0 | 5.2<br>5.3 | 5.2 | — |
| 1.6 | 9.2<br>8.8 | 9.0 | 3.8 |
| 8.0 | 30.0<br>28.7 | 29.4 | 24.2 |
| 40 | 138<br>126 | 132 | 127 |
| 200 | 761<br>774 | 768 | 763 |
| 1000 | 4203<br>4080 | 4142 | 4137 |
| 5000 | 17,357<br>17,080 | 17,218 | 17,213 |

### CALIBRATOR DILUENT RD6N




| (pg/mL) | RLU | Average | Corrected |
|---|---|---|---|
| 0 | 6.9<br>6.8 | 6.8 | — |
| 1.6 | 9.4<br>9.6 | 9.5 | 2.7 |
| 8.0 | 27.2<br>25.5 | 26.4 | 19.6 |
| 40 | 134<br>123 | 128 | 121 |
| 200 | 731<br>726 | 728 | 721 |
| 1000 | 4397<br>4337 | 4367 | 4360 |
| 5000 | 18,507<br>18,607 | 18,557 | 18,550 |

## 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.

### CELL CULTURE SUPERNATE ASSAY

| | Intra-Assay Precision | | | | Inter-Assay Precision | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| **n** | 20 | 20 | 20 | 20 | 40 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 2.38 | 10.1 | 125 | 1306 | 2.80 | 11.9 | 137 | 1400 |
| **Standard deviation** | 0.21 | 0.85 | 8.13 | 100 | 0.41 | 1.0 | 7.2 | 60 |
| **CV (%)** | 8.8 | 8.4 | 6.5 | 7.7 | 14.6 | 8.4 | 5.3 | 4.3 |

### SERUM/PLASMA ASSAY

| | Intra-Assay Precision | | | | Inter-Assay Precision | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| **n** | 20 | 20 | 20 | 20 | 40 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 3.63 | 18.9 | 177 | 1666 | 3.7 | 15.9 | 166 | 1547 |
| **Standard deviation** | 0.34 | 0.56 | 8.39 | 78.1 | 0.43 | 1.3 | 13.0 | 117 |
| **CV (%)** | 9.4 | 3.0 | 4.7 | 4.7 | 11.6 | 8.2 | 7.8 | 7.6 |

## RECOVERY

The recovery of recombinant human IL-4 spiked to three different levels in samples throughout the range of the assay was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=9) | 103 | 87-115% |
| Serum (n=6) | 93 | 76-109% |
| EDTA plasma (n=6) | 92 | 78-106% |
| Heparin plasma (n=6) | 92 | 82-102% |
| Citrate plasma (n=6) | 91 | 83-100% |

## SENSITIVITY

The minimum detectable dose (MDD) of IL-4 is typically less than 1.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU of twenty zero standard replicates and calculating the corresponding concentration.

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of IL-4 in various matrices were diluted with the appropriate 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) | Citrate plasma (n=5) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 99 | 105 | 105 | 105 | 102 |
| | Range (%) | 88-108 | 98-113 | 94-113 | 94-115 | 95-109 |
| 1:4 | Average % of Expected | 99 | 103 | 102 | 101 | 99 |
| | Range (%) | 86-117 | 90-121 | 89-121 | 85-112 | 88-110 |
| 1:8 | Average % of Expected | 98 | 100 | 101 | 97 | 98 |
| | Range (%) | 84-109 | 87-113 | 90-115 | 87-108 | 85-108 |
| 1:16 | Average % of Expected | 96 | 101 | 100 | 100 | 99 |
| | Range (%) | 87-109 | 85-113 | 92-110 | 85-111 | 83-108 |
| 1:32 | Average % of Expected | 95 | 96 | 97 | 98 | 98 |
| | Range (%) | 88-111 | 87-106 | 87-108 | 85-113 | 85-108 |

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-4 produced at R&D Systems.

The NIBSC/WHO IL-4 International Standard 88/656, intended as a potency standard, was evaluated in this kit. This standard contains CHO cell-expressed recombinant human IL-4. The dose response curve of the International Standard parallels the QuantiGlo standard curve. To convert sample values obtained with the QuantiGlo Human IL-4 kit to approximate NIBSC 88/656 International Units, use the equation below.

NIBSC (88/656) approximate value (IU/mL) = 0.0163 QuantiGlo Human IL-4 value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma** - Forty-five serum and plasma samples from apparently healthy volunteers were evaluated for the presence of IL-4 in this assay. No medical histories were available for the donors used in this study. One serum sample measured at 1.9 pg/mL. All other samples measured less than the lowest IL-4 standard, 1.6 pg/mL.

**Cell Culture Supernates:**
Human T cells (2 x $10^6$ cells/mL) were cultured in DMEM supplemented with 10% fetal calf serum and stimulated for 6 days with 5 µg/mL PHA and 10 ng/mL rhIL-2. Cells were then cultured in DMEM supplemented with 10% fetal calf serum, 10 ng/mL PMA, and 1 µg/mL calcium ionomycin, and stimulated for 20 hours with 5 µg/mL PHA. The cell culture supernate was removed, assayed for human IL-4, and measured 478 pg/mL.

HuT 78 human cutaneous T cell lymphoma cells (2 x $10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 10 ng/mL recombinant human IL-2 and 50 ng/mL PMA. Cells were then seeded into flasks pre-coated with 10 µg/mL mouse anti-human CD3. After 24 hours, the cell culture supernate was removed, assayed for human IL-4, and measured 287 pg/mL.

## SPECIFICITY

This assay recognizes natural and recombinant human IL-4.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5P (1X) and Calibrator Diluent RD6N and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range rhIL-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

Common γ Chain
GM-CSF
sgp130
IL-2 Rα
IL-3
IL-3 Rα
IL-4 Rα
IL-5
IL-5 Rα
IL-6 R
IL-7
IL-13
IL-13 Rα1
IL-13 Rα2

### Recombinant mouse:

Common γ Chain
GM-CSF
IL-3
IL-4
IL-4 Rα
IL-5
IL-6
IL-7
IL-13
IL-13 Rα1
IL-13 Rα2

### Other recombinants:

bovine IL-4
canine IL-4
cotton rat IL-4
equine IL-4
feline IL-4
porcine IL-4
rabbit IL-4
rat IL-4
rhesus macaque IL-4

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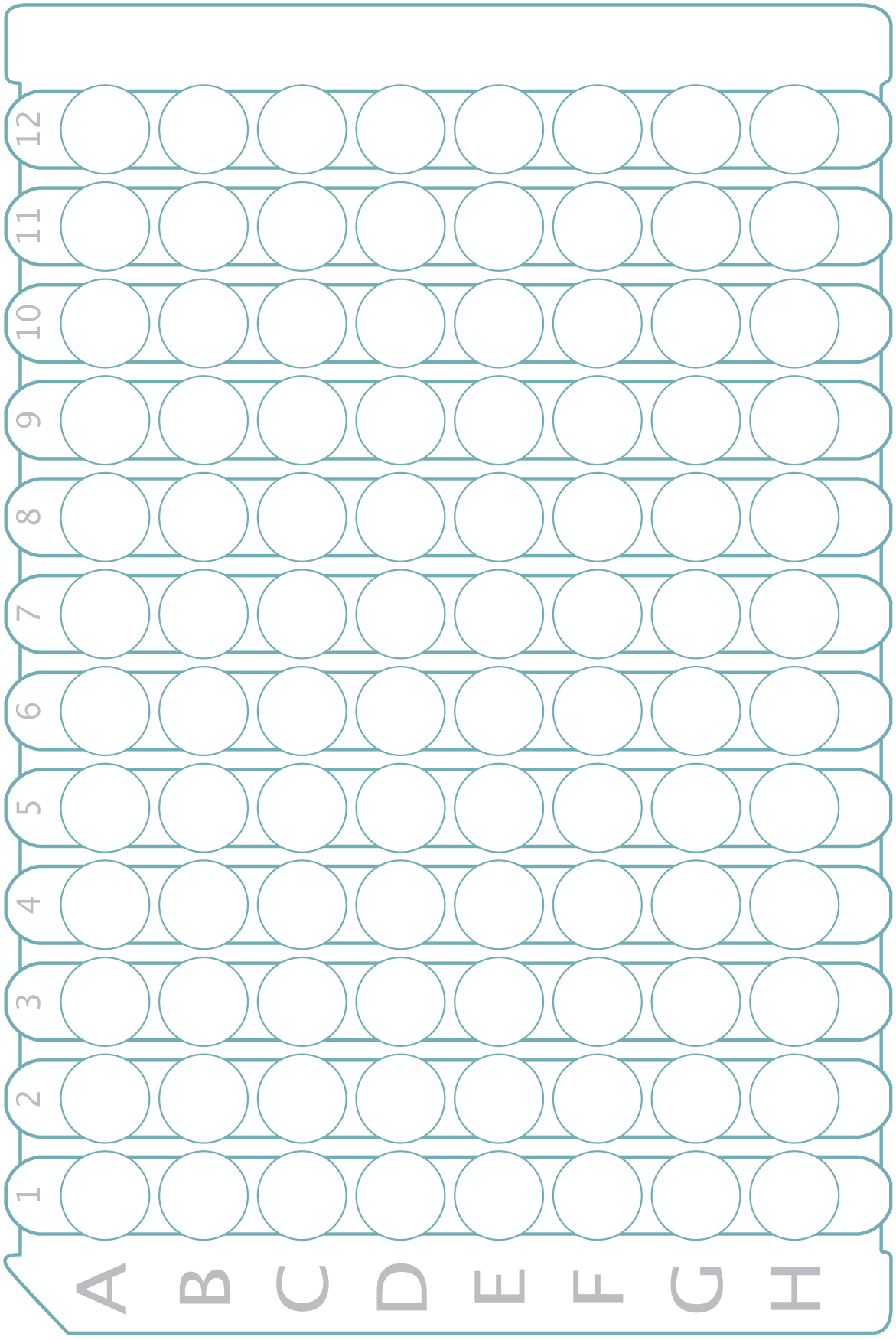
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



## NOTES