# Quantikine® ELISA

## Human LIF Immunoassay

Catalog Number DLF00B

For the quantitative determination of human Leukemia Inhibitory Factor (LIF) 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

Leukemia Inhibitory Factor (or LIF) is member of the IL-6 family of cytokines (1-2). It is a variably glycosylated, 38-67 kDa polypeptide originally identified as a proliferation inhibitor and differentiation inducer of the mouse M1 myeloid leukemia cell line (3-5). The mature LIF molecule measures 180 amino acid (aa) residues in length, with multiple potential N-linked and O-linked glycosylation sites plus six conserved cysteines that form three intramolecular disulfide bridges (3, 6, 7). Mature mouse LIF is 78% identical to human LIF at the aa sequence level (6, 7). Although such homology suggests high conservation of LIF biology, notable differences exist between the reported forms of mouse and human LIF proteins and their receptors. For example, alternative splice events are known to occur in mice, but not humans, creating two isoforms of secreted LIF (4). Additionally, three isoforms of the LIF receptor α-chain, two soluble forms and one transmembrane form, have been reported in mice (8, 9), but not in humans.

LIF binds to the LIF receptor (LIF R), a member of the hematopoietin superfamily of receptors (10). When expressed alone, LIF R binds LIF with low affinity (Kd = 1-3 nM) (11, 12). However, when expressed with LIF gp130, the 130 kDa "affinity-converting" signal-transducing subunit common to IL-6 family receptor complexes, a high-affinity tripartite complex forms between LIF R, LIF, and gp130 (Kd = 10-200 pM) (11-14). This triggers the downstream signaling events through the JAK/STAT, PI3K, and MAPK signaling pathways (15-18).

Cells known to express LIF include activated T-cells, monocytes, and astrocytes (4), osteoblasts (19), keratinocytes (20), regenerating skeletal muscle (21), mast cells (22), and fibroblasts (4, 23). Functionally, LIF has been implicated in a number of processes including stem cell maintenance, reproduction, development, hematopoiesis, bone metabolism, skeletal muscle regeneration, and inflammation (1, 5, 24). Research also suggests LIF plays a role in protecting myocardium cells during ischemia-reperfusion and increases the brain's ability to repair after an ischemic stroke by protecting neuronal cells from reactive oxygen species and maintaining high populations of neuronal stem cells (25, 26). LIF has been detected in a variety of body fluids. Elevated concentrations (pg/mL) of LIF in serum have been correlated with the presence of hematologic malignancy (lymphoma) (27), while elevated levels in bronchoalveolar lavage have been correlated with an increase in markers of inflammation (28). Elevated concentrations (ng/mL) of LIF have been correlated with the peripheral white cell count in rheumatoid arthritis patients (29).

The Quantikine® Human LIF Immunoassay is a 4.5 hours solid phase ELISA designed to measure human LIF in cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant human LIF and antibodies raised against the recombinant protein. Results obtained for naturally occurring human LIF showed linear curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human LIF.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human LIF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LIF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme linked polyclonal antibody specific for human LIF 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 LIF 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- 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 # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Human LIF Microplate** | 899060 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human LIF. | 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 LIF Standard** | 899062 | 2 vials of recombinant human LIF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Discard after use. Use a fresh standard for each assay. |
| **Human LIF Conjugate** | 899061 | 21 mL of a polyclonal antibody specific for human LIF conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-18** | 895202 | 12 mL of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5-3** | 895436 | 21 mL of a buffered protein base 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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human LIF Controls (optional; R&D Systems®, Catalog # QC256).

## 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 SDS 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 hemolyzed samples are not suitable for use in this assay.*

## SAMPLE PREPARATION

Cell culture supernates may need a dilution due to high endogenous levels. Multiple dilutions are recommended for unknown samples.

## REAGENT PREPARATION

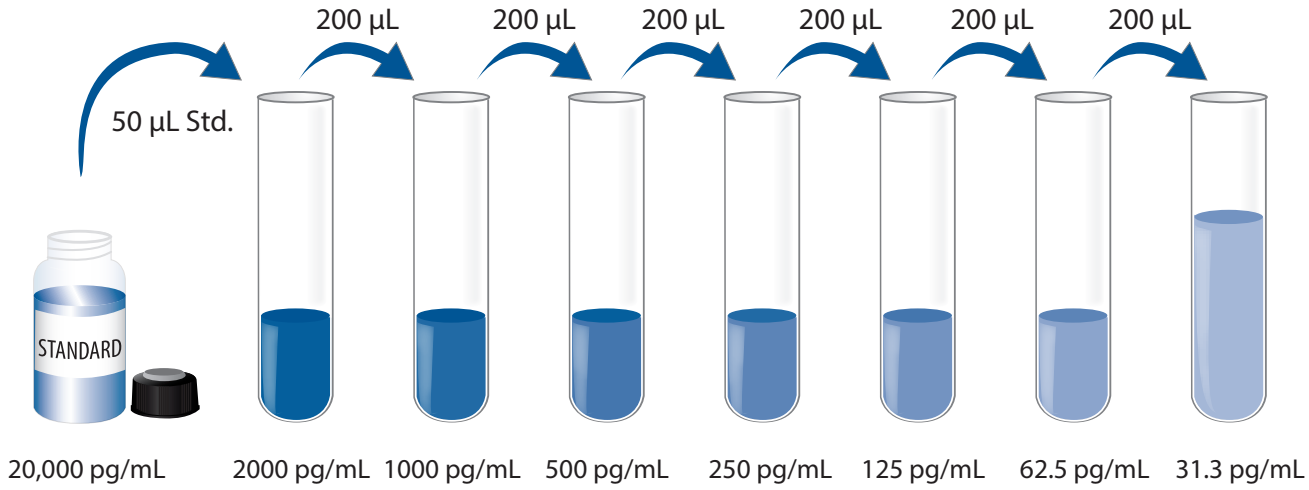
**Bring all reagents to room temperature before use.**

**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 480 mL of 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 LIF Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human LIF Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution by inverting vials and allow the standard to sit on benchtop for a minimum of 15 minutes.
**Note:** *Do not vortex.*

Pipette 450 µL of Calibrator Diluent RD5-3 into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 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, controls, and samples 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 50 µL of Assay Diluent RD1-18 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a benchtop. A plate layout is provided to record standards and samples assayed.

   **Note:** *Samples must be pipetted within 15 minutes.*
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 Human LIF Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on a benchtop.
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. **Protect from light.**
9. 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.
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

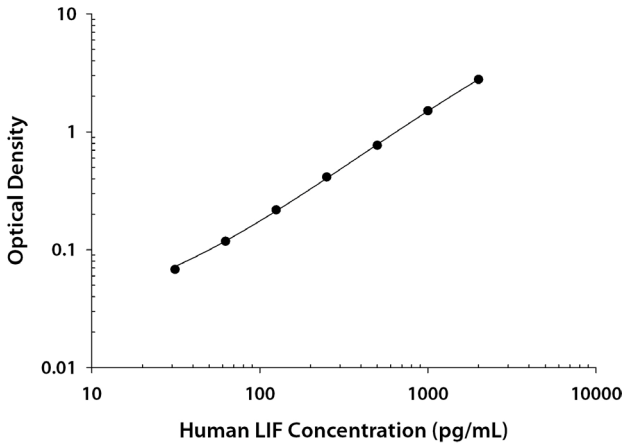
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 LIF 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, 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 | 0.018<br>0.018 | 0.018 | — |
| 31.3 | 0.067<br>0.068 | 0.068 | 0.050 |
| 62.5 | 0.117<br>0.119 | 0.118 | 0.100 |
| 125 | 0.214<br>0.221 | 0.218 | 0.200 |
| 250 | 0.403<br>0.425 | 0.414 | 0.396 |
| 500 | 0.750<br>0.786 | 0.768 | 0.750 |
| 1000 | 1.480<br>1.535 | 1.508 | 1.490 |
| 2000 | 2.773<br>2.784 | 2.779 | 2.761 |

## 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. 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 | 20 | 20 | 20 |
| **Mean (pg/mL)** | 255 | 778 | 1433 | 268 | 827 | 1548 |
| **Standard deviation** | 11.0 | 21.8 | 32.6 | 21.0 | 50.9 | 76.7 |
| **CV (%)** | 4.3 | 2.8 | 2.3 | 7.8 | 6.2 | 5.0 |

## RECOVERY

The recovery of human LIF spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 103 | 92-112% |
| Serum (n=4) | 92 | 83-101% |
| EDTA plasma (n=4) | 90 | 83-96% |
| Heparin plasma (n=4) | 90 | 83-99% |

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human LIF were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernate (n=4) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 99 | 98 | 98 | 99 |
| | Range (%) | 91-108 | 91-104 | 91-110 | 93-105 |
| 1:4 | Average % of Expected | 99 | 105 | 105 | 105 |
| | Range (%) | 95-104 | 102-113 | 100-113 | 96-109 |
| 1:8 | Average % of Expected | 97 | 107 | 105 | 105 |
| | Range (%) | 93-105 | 101-113 | 98-111 | 92-113 |
| 1:16 | Average % of Expected | 93 | 113 | 106 | 108 |
| | Range (%) | 85-106 | 104-118 | 100-114 | 98-118 |

## SENSITIVITY

Thirty-six assays were evaluated and the minimum detectable dose (MDD) of human LIF ranged from 0.734-5.36 pg/mL. The mean MDD was 2.07 pg/mL.

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

## CALIBRATION

This immunoassay is calibrated against a highly purified HEK293-expressed recombinant human LIF.

The NIBSC/WHO LIF (human rDNA derived) Reference Reagent 93/562 was evaluated in this kit. The dose response curve of the NIBSC standard 93/562 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human LIF kit to approximate NIBSC 93/562 Units, use the equation below.

NIBSC/WHO (93/562) approximate value (U/mL) = 0.0063 x Quantikine Human LIF value (pg/mL)

**Note:** *Based on data generated April 2019.*

## SAMPLE VALUES

**Serum** - Thirty serum samples from apparently healthy volunteers were evaluated for the presence of human LIF in this assay. No detectable levels were observed. No medical histories were available for the donors used in this study.

**Cell Culture Supernates** - Human peripheral blood mononuclear cells (1 x $10^6$ cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 5 days. Aliquots of the cell culture supernates were removed, assayed for levels of natural human LIF, and measured 267 pg/mL and 3205 pg/mL, respectively.

A375 human melanoma cells were cultured in DMEM supplemented with 10% fetal bovine serum until nearly confluent. Cells were then washed with PBS and switched to serum-free media. Cells were cultured untreated or treated with 1000 pM of recombinant human Oncostatin M (R&D Systems®, Catalog # 295-OM/CF) and 100 pM of recombinant human TNF-α (R&D Systems®, Catalog # 210-TA/CF) for 24 hours. Aliquots of the cell culture supernates were removed, assayed for levels of natural human LIF, and measured 1648 pg/mL and 18,019 pg/mL, respectively.

## SPECIFICITY

This assay recognizes natural and recombinant human LIF.

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

### Recombinant human:

Cardiotrophin-1
CNTF
CNTF Rα
DCBLD2
G-CSF
GM-CSF
gp130
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-6/IL-6 Rα Complex
IL-6
IL-6 Rα
IL-7
IL-8
IL-11
IL-11 Rα
LIF Rα
Oncostatin M
Oncostatin M Rβ
TNF-α
TNF-β

### Recombinant mouse:

IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
GM-CSF
LIF

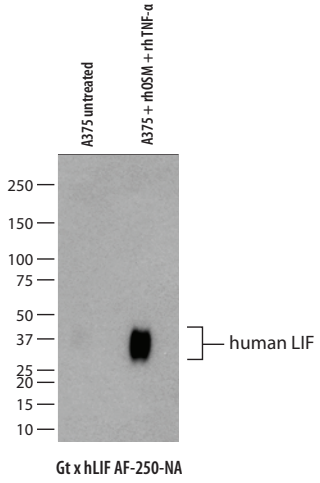
### Recombinant rat:

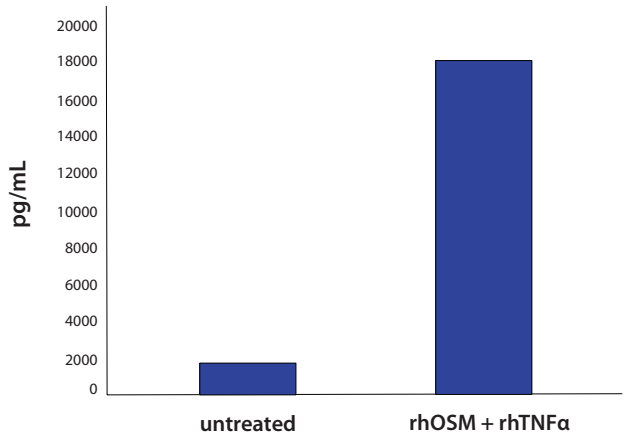
LIF

### Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
human TGF-β1
porcine PDGF
porcine TGF-β2

Recombinant canine LIF does not interfere but does cross-react approximately 13.1% in this assay.




Conditioned media samples were analyzed by Western Blot and Quantikine® ELISA. A375 cells were switched to serum free media and then left untreated or treated with 1000 pM of recombinant human (rh) Oncostatin M (R&D Systems®, Catalog # 295-OM/CF) and 100 pM of rhTNF-α (R&D Systems®, Catalog # 210-TA/CF) for 24 hours prior to collecting conditioned media. For Western blot, samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with goat anti-hLIF (R&D Systems®, Catalog # AF-250-NA). The Western Blot shows a direct correlation with ELISA values for these samples.

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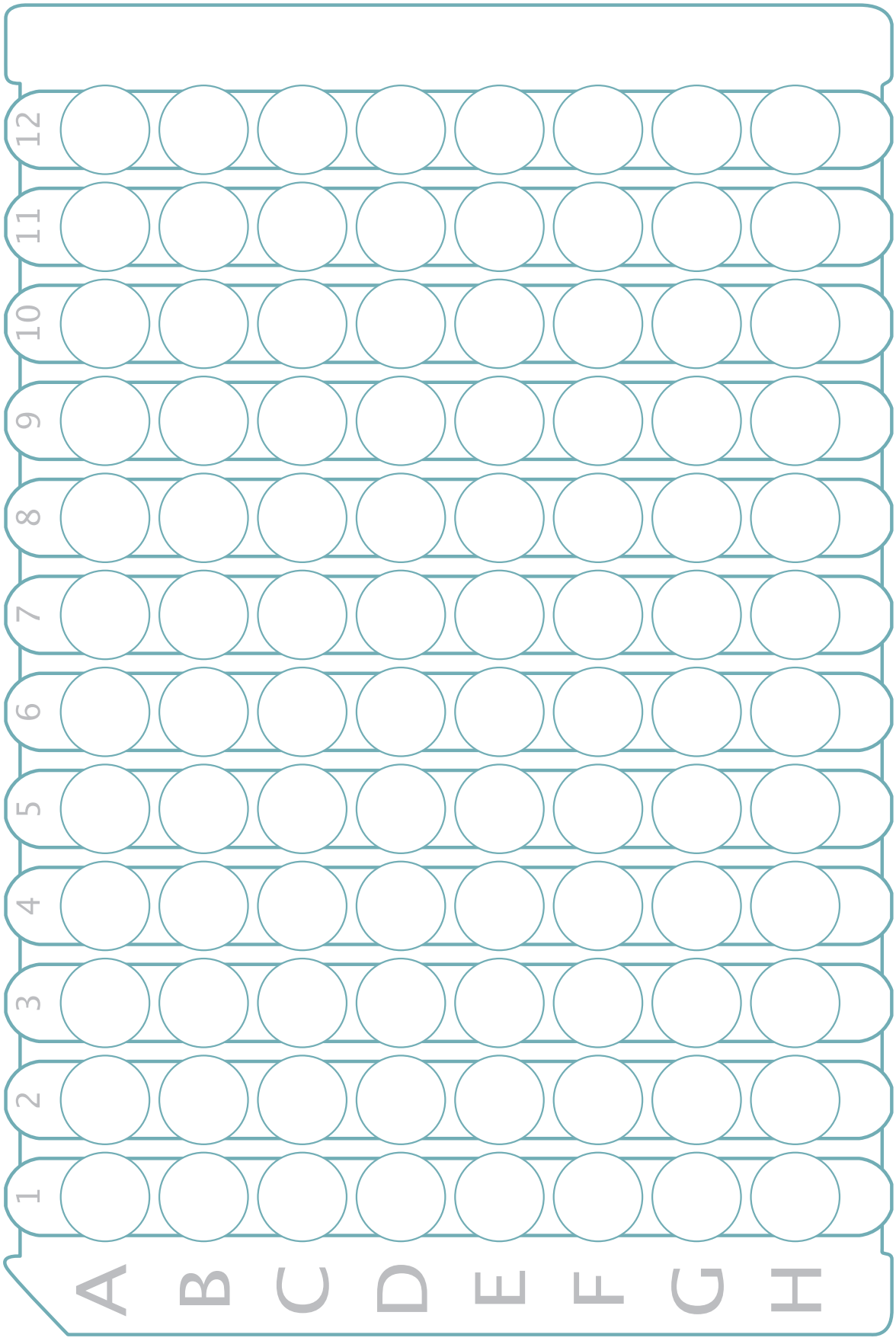
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# NOTES