# Quantikine® ELISA

## Mouse/Rat IL-17F Immunoassay

Catalog Number M17F0

For the quantitative determination of mouse and rat Interleukin 17F (IL-17F) 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-17F (IL-17F), also known as ML-1, is a member of the IL-17 family of cysteine knot cytokines which are involved in inflammation and tissue remodeling (1, 2). IL-17F is secreted as a 45 kDa disulfide-linked homodimer and as an approximately 40 kDa disulfide-linked heterodimer with IL-17A (IL-17A/F) (3-5). Mature mouse IL-17F shares 56% and 90% amino acid identity with human and rat IL-17F, respectively. IL-17F and IL-17A are co-expressed by the Th17 subset of activated $CD4^+$ T cells as well as by mast cells, basophils, monocytes, and B cells (6-9). The expression of IL-17F is induced by IL-23 and can be suppressed by IL-17A (5, 7, 10).

IL-17F binds and induces signaling through the widely expressed receptors IL-17 RA and IL-17 RC. These proteins form a ligand-induced receptor complex for IL-17A, IL-17F, and IL-17A/F (4, 11-13). IL-17 RC is required for signaling in response to all three cytokines (11). IL-17F triggers the production of multiple inflammatory cytokines from endothelial cells, epithelial cells, and keratinocytes (8, 14, 15). These inflammatory cytokines induce neutrophil infiltration and activation (16, 17). IL-17A/F is less potent than the IL-17A homodimer at IL-17 receptor activation and more potent than the IL-17F homodimer (5). IL-17 family cytokines play a role in a number of inflammatory diseases such as rheumatoid arthritis, asthma, systemic lupus erythematosus, and allograft rejection (18). IL-17F is upregulated in allergic asthma and psoriasis, and it contributes to allergic inflammation and airway remodeling (17, 19). IL-17F also promotes osteoclast and osteoblast maturation (11, 20).

The Quantikine Mouse/Rat IL-17F Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse and rat IL-17F in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-17F and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse or rat IL-17F 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 naturally occurring mouse and rat IL-17F.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat IL-17F has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse or rat IL-17F present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat IL-17F 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 or rat IL-17F 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 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.
- 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 & 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 |
|---|---|---|---|
| **Mouse/Rat IL-17F Microplate** | 894900 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat IL-17F. | 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.* |
| **Mouse/Rat IL-17F Standard** | 894902 | 2 vials of recombinant mouse IL-17F in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Discard after use.<br>Use a fresh Standard and Control for each assay. |
| **Mouse/Rat IL-17F Control** | 894903 | 2 vials of recombinant mouse IL-17F in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label. | |
| **Mouse/Rat IL-17F Conjugate** | 894901 | 12 mL of a polyclonal antibody specific for mouse/rat IL-17F conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-40** | 895513 | 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** | 895174 | 23 mL of diluted hydrochloric 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.

## 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. 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Mouse Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Rat Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Mouse 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.

**Rat Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 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 samples have not been validated for use in this assay.*

## REAGENT PREPARATION

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

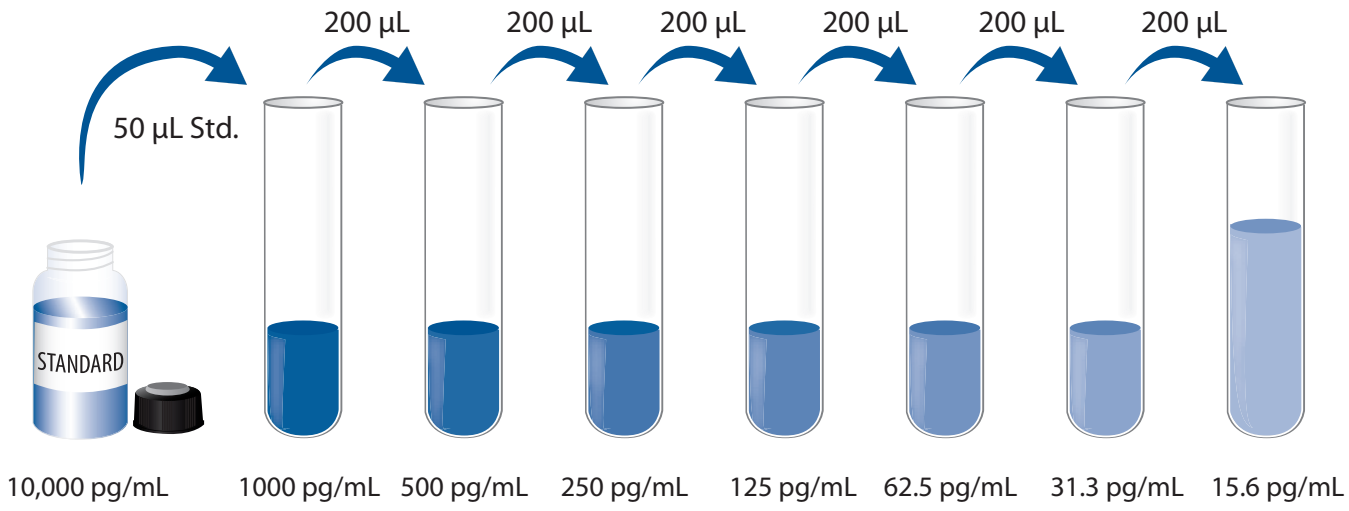
**Mouse/Rat IL-17F Control** - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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. 100 µL of the resultant mixture is required per well.

**Mouse/Rat IL-17F Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat IL-17F Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-3 into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. 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 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, Control, and samples 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, and reseal.
3. Add 50 µL of Assay Diluent RD1-40 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 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 100 µL of Mouse/Rat IL-17F 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. 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.

## CALCULATION OF RESULTS

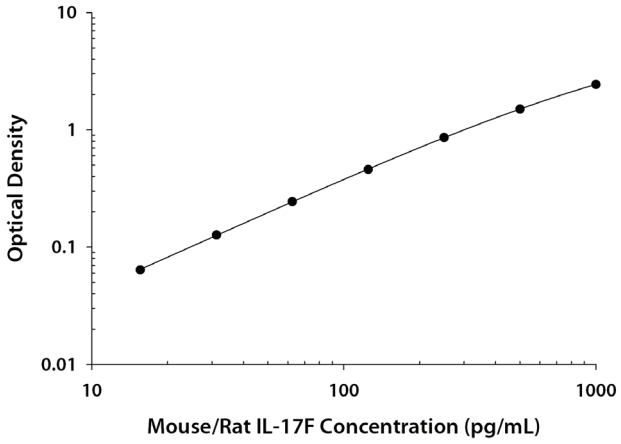
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat IL-17F 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 ia provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.009<br>0.009 | 0.009 | — |
| 15.6 | 0.073<br>0.073 | 0.073 | 0.064 |
| 31.3 | 0.134<br>0.138 | 0.136 | 0.127 |
| 62.5 | 0.252<br>0.253 | 0.253 | 0.244 |
| 125 | 0.466<br>0.471 | 0.469 | 0.460 |
| 250 | 0.861<br>0.875 | 0.868 | 0.859 |
| 500 | 1.508<br>1.515 | 1.512 | 1.503 |
| 1000 | 2.401<br>2.493 | 2.447 | 2.438 |

## 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)** | 56.7 | 211 | 409 | 53.2 | 202 | 399 |
| **Standard deviation** | 1.26 | 4.93 | 13.1 | 3.98 | 11.4 | 20.2 |
| **CV (%)** | 2.2 | 2.3 | 3.2 | 7.5 | 5.6 | 5.1 |

## RECOVERY

The recovery of mouse IL-17F spiked to levels throughout the range of the assay in various matrices was evaluated.

| Mouse Samples | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 96 | 92-99% |
| Serum (n=4) | 95 | 83-109% |
| EDTA plasma (n=4) | 107 | 101-118% |
| Heparin plasma (n=4) | 101 | 88-121% |

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse IL-17F in each matrix were diluted with Calibrator Diluent and assayed.

| Mouse Samples | | Cell culture media (n=4) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 102 | 105 | 104 | 104 |
| | Range (%) | 101-104 | 101-107 | 103-106 | 101-107 |
| 1:4 | Average % of Expected | 101 | 109 | 104 | 108 |
| | Range (%) | 99-103 | 103-113 | 103-105 | 106-110 |
| 1:8 | Average % of Expected | 101 | 113 | 105 | 108 |
| | Range (%) | 99-102 | 105-117 | 102-106 | 105-110 |
| 1:16 | Average % of Expected | 102 | 113 | 106 | 110 |
| | Range (%) | 100-106 | 106-117 | 105-107 | 105-116 |

**Note:** *Rat samples were evaluated and no significant difference in recovery or linearity was observed from the data above.*

## SENSITIVITY

Thirty-five assays were evaluated and the minimum detectable dose (MDD) of mouse/rat IL-17F ranged from 0.214-1.74 pg/mL. The mean MDD was 0.532 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 mouse IL-17F produced at R&D Systems.

## SAMPLE VALUES

**Serum/Plasma** - Samples were evaluated for the presence of mouse/rat IL-17F in this assay.

| Mouse Samples | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Serum (n=10) | 243 | 40 | ND-602 |
| EDTA plasma (n=5) | ND | 0 | ——— |
| Heparin plasma (n=5) | 170 | 20 | ND-170 |

ND=Non-detectable

**Note:** *The same number of rat serum and plasma samples were assayed and no detectable levels were observed.*

**Cell Culture Supernates:**

Mouse $CD4^+$ cells were isolated from splenocytes using a MagCellect Mouse IL-2 Isolation Kit (R&D Systems, Catalog # MAGM202). Cells were cultured in basal media RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate. For Th17, cells were plated on a flask coated with 2 µg/mL of goat anti-mouse IgG followed by 5 µg/mL of anti-mouse CD3. Basal media was supplemented with 1 µg/mL of anti-mouse CD28, 10 µg/mL anti-mouse IL-4, 10 µg/mL of anti-mouse IFN-γ, 1 ng/mL of recombinant human TGF-β1, and 100 ng/mL of recombinant mouse IL-6. Th17 cells were cultured for 4 days and then 50 nM PMA and 500 ng/mL ionomycin was added for 4 hours prior to harvest. Aliquots of the cell culture supernates were removed, assayed for mouse IL-17F, and measured 36.2 pg/mL and 224 pg/mL, respectively.

Spleens from rats were removed, rinsed in PBS, and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing DME supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL of streptomycin sulfate, and 50 mg/mL gentamicin solution. Cells were culture unstimulated and stimulated with 50 ng/mL of recombinant rat IL-2 and 50 mg/mL PHA for 3 or 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of rat IL-17F.

| Condition | Day 3 (pg/mL) | Day 6 (pg/mL) |
|---|---|---|
| Unstimulated | ND | 237 |
| Stimulated | 129 | 394 |

ND=Non-detectable

## SPECIFICITY

This assay recognizes natural and recombinant mouse and rat IL-17F.

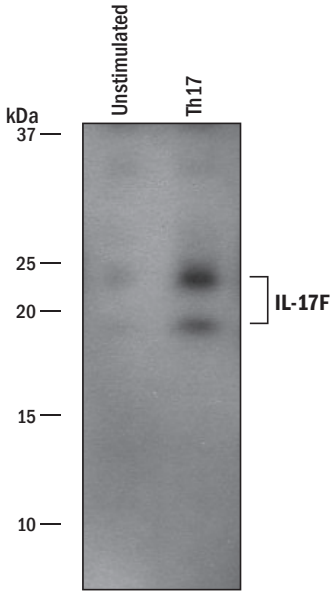
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat IL-17F control were assayed for interference. No significant cross-reactivity or interference was observed.

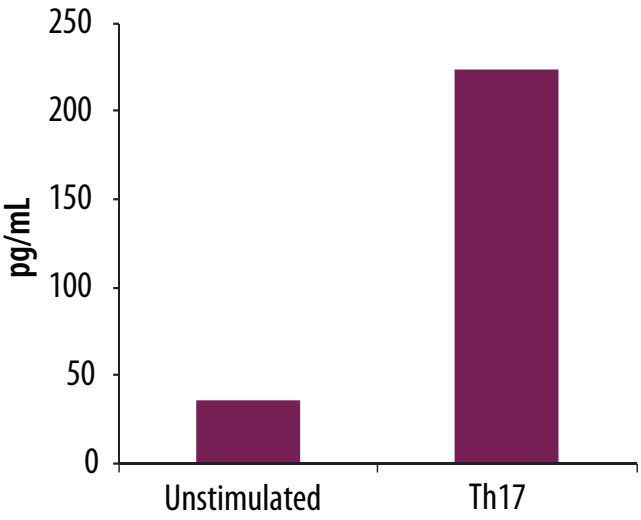
**Recombinant mouse:**
IL-17/IL-17A

**Recombinant rat:**
IL-17A
IL-17E
IL-17F

**Recombinant human:**
IL-17F

Recombinant mouse IL-17A/IL-17F Heterodimer cross-reacts approximately 9% in this assay.




Mouse CD4$^+$ T cells were isolated using MAGM202, and left unstimulated or cultured to promote Th17 differentiation. Conditioned media were collected and analyzed by Western blot and Quantikine ELISA. For the Western blot, media were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody in this kit. The Western blot shows a direct correlation with the ELISA value for these samples.

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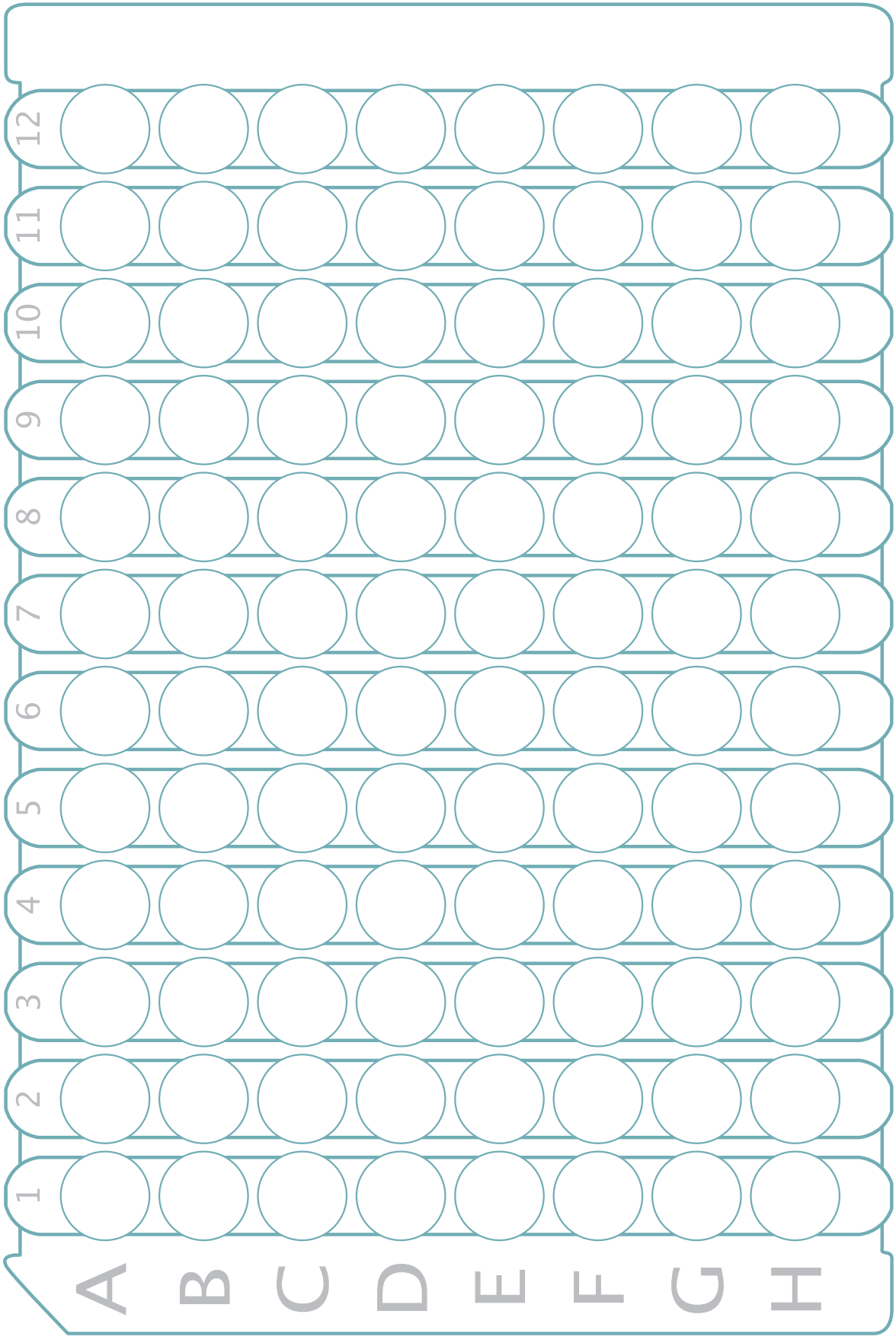
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# NOTES

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