# Quantikine™ ELISA

## Human FABP2/I-FABP Immunoassay

Catalog Number DFBP20

For the quantitative determination of human Fatty Acid Binding Protein 2 (FABP2) concentrations in cell culture supernates, cell lysates, serum, plasma, and urine.

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

Fatty acid binding protein-2 (FABP2), also known as intestinal (I)-FABP, is a 15 kDa member the FABP family of cytoplasmic lipid chaperones (1-3). FABP2 is primarily expressed in small intestinal enterocytes along with FABP1. Intestinal FABPs are thought to be involved in the uptake, metabolism, and transport of dietary long-chain fatty acids (4, 5). Mature human FABP2 is 132 amino acids (aa) in length and contains a flattened barrel structure composed of antiparallel β-strands and two α-helices. The tertiary structure of FABP2 is conserved across all FABPs, despite the aa sequences between any two FABP family members are highly divergent. Mature human FABP2 shares 33% and 24% aa identity with human FABP1 and FABP3, respectively. In contrast, FABP2 is highly similar across species. Human FABP2 shares 78%, 82%, and 86% sequence identity with mouse, rat, and canine FABP2, respectively.

The preferred ligands for FABP2 are sixteen- to twenty-chain saturated and unsaturated fatty acids (6). Unlike FABP1, which has two high-affinity ligand binding sites, FABP2 has a single, low-affinity ligand binding site (7, 8). FABP2 facilitates the processing of fatty acids into triglycerides by transporting them from the plasma membrane into the endoplasmic reticulum (2). This is accomplished through a collisional transfer mechanism that requires the direct interaction of FABP2 with the acceptor or donor membrane (7, 9). The α-helical regions of FABP2 are thought to be involved in protein-membrane interactions that facilitate the delivery and removal of fatty acids from the lipid bilayer (10). It is suggested that the fatty acid binds to the outside of the molecule, and this binding subsequently induces a conformational change in the binding protein, resulting in internalization of the ligand (2). Due to compensatory actions of FABP1 in enterocytes, FABP2 is not essential for fat absorption under normal dietary conditions. Under dietary restriction however, FABP2 is implicated in fatty acid metabolic dysfunction. This affect is also thought to be gender specific (11).

Under normal physiological conditions FABP2 is detectable in serum at low levels (12). Changes in serum FABP2 are associated with intestinal damage, including coeliac disease and ischemia (13-16). FABP2 does not contain a secretory signal sequence, so it enters the circulation following damage to the intestinal epithelium (12). FABP2 levels in serum are associated with the onset of HIV-associated immune reconstitution inflammatory syndrome (17). A polymorphism in FABP2 resulting in the substitution of alanine (A) with threonine (T) at codon 54 (A54T) increases the affinity of FABP2 for long-chain fatty acids. The A54T polymorphism is associated with metabolic disorders including insulin resistance, high obesity, and type II diabetes (2, 3).

The Quantikine™ Human FABP2/I-FABP Immunoassay is a 4.5 hour solid phase ELISA designed to measure human FABP2 levels in cell culture supernates, cell lysates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human FABP2 and antibodies raised against the recombinant protein. Results obtained for naturally occurring human FABP2 showed linear curves that were parallel to the standard curves obtained using the Quantikine FABP2 kit standards. These results indicate that this kit can be used to determine relative mass values for natural human FABP2.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FABP2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FABP2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human FABP2 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 FABP2 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.
- If samples generate values higher than the highest standard, further dilute the samples with 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 FABP2 Microplate** | 894979 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human FABP2. | 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 FABP2 Standard** | 894981 | 2 vials of recombinant human FABP2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Use a new standard for each assay. Discard after use. |
| **Human FABP2 Conjugate** | 894980 | 21 mL of a polyclonal antibody specific for human FABP2 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-63** | 895179 | 12 mL of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5-5** | 895485 | 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 2N 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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human FABP2 Controls (optional; R&D Systems®, Catalog # QC213)

## SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems®, Catalog # 890713).

## 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.

**Cell Lysates** - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

**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.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-5.

## CELL LYSIS PROCEDURE

**Use the following procedure for the preparation of cell lysate samples.**

1. Wash cells three times in cold PBS.
2. Resuspend cells at 1 x $10^7$ cells/mL in Cell Lysis Buffer 1.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at 8000 x g for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at ≤ -70 °C until ready for use.

## REAGENT PREPARATION

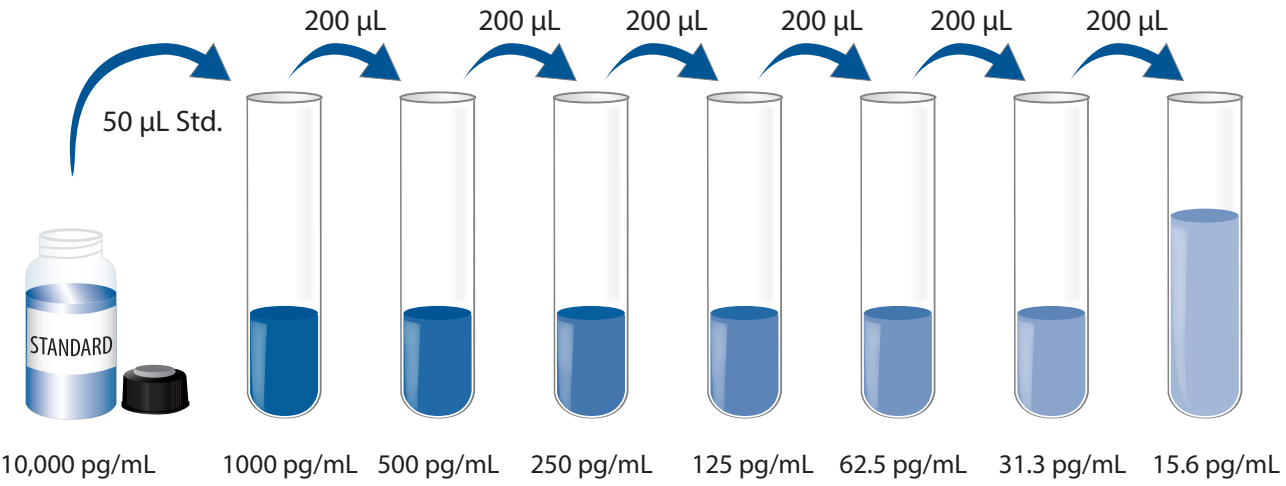
**Bring all reagents to room temperature before use.**

**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 FABP2 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FABP2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-5 into the 1000 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 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-5 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 RD1-63 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 200 µL of Human FABP2 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. 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.

*Samples may require dilution. See the Sample Preparation section.

## CALCULATION OF RESULTS

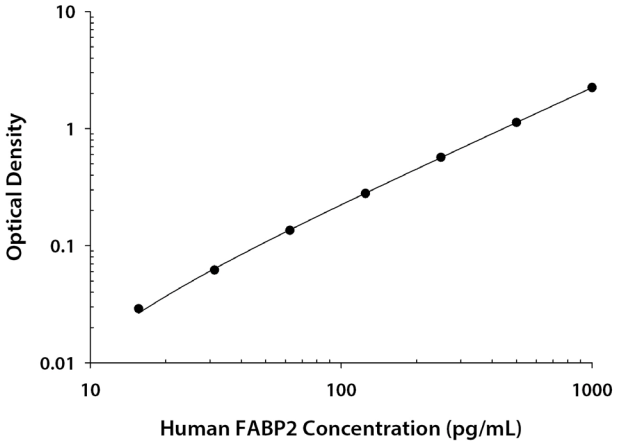
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 FABP2 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 | 0.048<br>0.052 | 0.050 | — |
| 15.6 | 0.077<br>0.081 | 0.079 | 0.029 |
| 31.3 | 0.110<br>0.114 | 0.112 | 0.062 |
| 62.5 | 0.181<br>0.188 | 0.185 | 0.135 |
| 125 | 0.325<br>0.333 | 0.329 | 0.279 |
| 250 | 0.618<br>0.619 | 0.619 | 0.569 |
| 500 | 1.169<br>1.180 | 1.175 | 1.125 |
| 1000 | 2.230<br>2.242 | 2.236 | 2.186 |

## 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)** | 77.5 | 207 | 425 | 80.5 | 216 | 446 |
| **Standard deviation** | 3.14 | 7.24 | 12.4 | 8.94 | 17.4 | 26.6 |
| **CV (%)** | 4.1 | 3.5 | 2.9 | 11.1 | 8.1 | 6.0 |

## RECOVERY

The recovery of human FABP2 spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 100 | 96-104% |
| Cell Lysis Buffer (n=2) | 83 | 81-85% |
| Serum (n=4) | 101 | 97-106% |
| EDTA plasma (n=4) | 97 | 85-102% |
| Heparin plasma (n=4) | 95 | 96-104% |
| Urine (n=4) | 90 | 85-95% |

## SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of human FABP2 ranged from 2.12-6.21 pg/mL. The mean MDD was 3.63 pg/mL.

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

## CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-derived recombinant human FABP2 produced at R&D Systems®.

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human FABP2 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernate (n=4) | Cell lysate (n=1) | Serum* (n=4) | EDTA plasma* (n=4) | Heparin plasma* (n=4) | Urine (n=4) |
|---|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 102 | 103 | 99 | 99 | 102 | 102 |
| | Range (%) | 102-103 | ——— | 97-103 | 95-104 | 92-109 | 99-105 |
| 1:4 | Average % of Expected | 102 | 103 | 96 | 99 | 103 | 97 |
| | Range (%) | 99-108 | ——— | 89-100 | 93-106 | 89-111 | 90-104 |
| 1:8 | Average % of Expected | 102 | 104 | 93 | 96 | 95 | 98 |
| | Range (%) | 101-104 | ——— | 83-105 | 84-106 | 82-110 | 97-101 |
| 1:16 | Average % of Expected | 105 | 115 | 92 | 99 | 103 | 94 |
| | Range (%) | 102-112 | ——— | 80-102 | 89-104 | 94-108 | 88-101 |

*Samples were diluted prior to assay.

## SAMPLE VALUES

**Serum/Plasma/Urine** - Samples from apparently healthy volunteers were evaluated for the presence of human FABP2 in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (pg/mL) | Range (pg/mL) | Standard Deviation (pg/mL) |
|---|---|---|---|
| Serum (n=36) | 1015 | 389-2129 | 455 |
| EDTA plasma (n=36) | 952 | 377-2049 | 444 |
| Heparin plasma (n=36) | 797 | 230-1800 | 381 |

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Urine (n=10) | 184 | 90 | ND-632 |

ND=Non-detectable

**Cell Culture Supernates** - Human peripheral blood leukocytes (PBL) were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were then cultured unstimulated or stimulated with 10 µg/mL PHA for 1 or 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human FABP2. No detectable levels were observed.

**Cell Lysates** - LoVo human colorectal adenocarcinoma cells were cultured in Kaighn's supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 1.5 g/L sodium bicarbonate and grown until confluent. An aliquot of the cell lysate was removed, assayed for human FABP2, and measured 82.3 pg/mg.

## SPECIFICITY

This assay recognizes natural and recombinant human FABP2.

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 FABP2 control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant human:**

FABP1
FABP3
FABP4
FABP5
FABP7
FABP8
FABP9
PPARγ
PPARγ1
PPARγ2

**Other factor:**

Linoleic Acid

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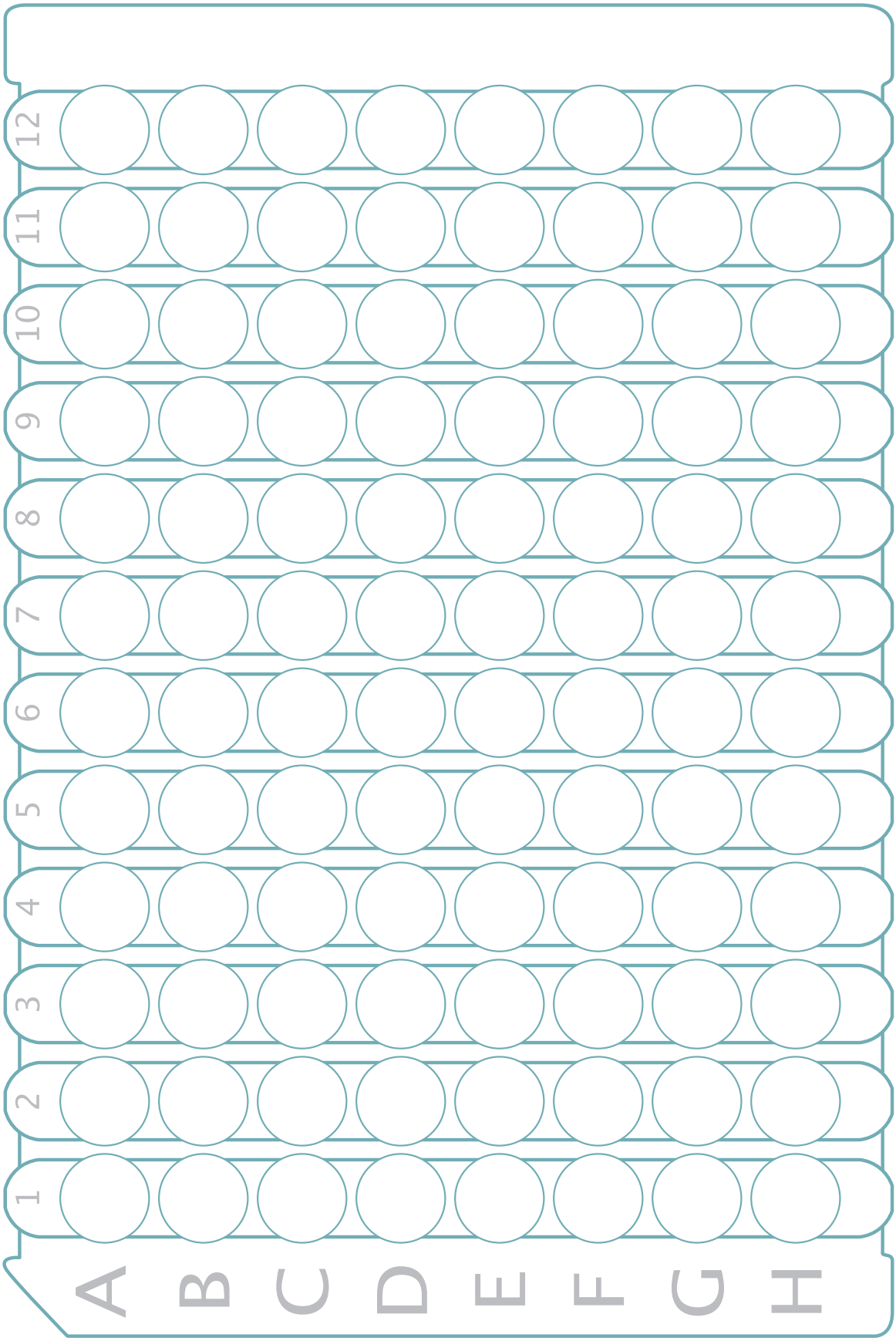
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



## NOTES