Magnetic Luminex® Performance Assay

Human Kidney Biomarker Premixed Kit

Catalog Number FCSTM16

For the simultaneous quantitative determination of multiple human kidney biomarker concentrations in 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

The kidneys play important roles in organismal homeostasis by regulating osmolality and blood pressure, aiding in the reabsorption of water and nutrients, excreting wastes, and secreting hormones. Renal function is also important in the metabolism and excretion of drugs (1). Therefore, analyzing nephrotoxicity using renal markers is an important experimental step during drug development. Historically, renal function has been evaluated by measuring serum creatinine and blood urea nitrogen levels (2). Recently, more sensitive kidney biomarkers have been identified and renal function can be assessed contextually by analyzing multiple proteins simultaneously. In addition, renal markers can be used to assess kidney development during embryogenesis as well as pathological conditions such as renal failure and renal cell carcinoma (2-6).

This kit can be used to simultaneously assess the levels of multiple kidney biomarkers in a single sample. For ease of use, the microparticles and the biotinylated detection antibodies are premixed in respective vials.

Analyte	Microparticle Region	Performance Data Online (www.RnDSystems.com/pdf/...)
Clusterin	20	LHK2937.pdf
Cystatin C	19	LHK1196.pdf
CXCL10/IP-10	25	LHK266.pdf
Lipocalin-2/NGAL	27	LHK1757.pdf
Osteopontin (OPN)	28	LHK1433.pdf
RBP4	29	LHK3378.pdf
TFF3	30	LHK4407.pdf
TIM-1/KIM-1/HAVCR*	26	LHK1750.pdf

*This *product is covered by one or more of the following US Patents: 7,300,652; 7,041,290; 6,664,385; and other US and foreign* patents pending or issued.

PRINCIPLE OF THE ASSAY

Magnetic Luminex® Performance Assay multiplex kits are designed for use with the Luminex® MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex® MAGPIX® Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED excites the dyes inside each microparticle to identify the region and the second LED excites the PE to measure the amount of analyte bound to the microparticle. A sample from each well is imaged with a CCD camera with a set of filter to differentiate excitation levels.

Analysis with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex uses one laser to excite the dyes inside each microparticle to identifying the microparticle region and the second laser to excite the PE to measure the amount of analyte bound to the microparticle. All excitation emitted as each microparticle passes through the flow cell is then analyzed to differentiate excitation levels using a Photomultiplier Tube (PMT) and an Avalanche Photodiode.

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 fall outside the dynamic range of the assay, further 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 these factors have been tested in the Magnetic Luminex® Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex® Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit. Refer to the enclosed certificate of analysis for specific analytes included in this kit.**

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, DILUTED, OR RECONSTITUTED MATERIAL
Kidney Biomarker Standard Cocktail	894311	2 vials of recombinant human kidney biomarkers in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard for each assay.
Human Kidney Biomarker Premixed Kit Magnetic Microparticle Cocktail	894641	1.2 mL of a concentrated microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Once diluted, 1X solutions must be discarded. Use fresh diluents for each assay.*
Human Kidney Biomarker Premixed Kit Biotin-Ab Cocktail	894642	1.2 mL of a concentrated biotinylated antibody cocktail with preservatives.	
Streptavidin-PE	892525	0.07 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Microparticle Diluent	895529	2 vials (6 mL/vial) of a buffered protein base with blue dye and preservative.	May be stored for up to 1 month at 2-8 °C.*
Diluent RD2-1	895970	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-62	895986	21 mL of a concentrated buffered animal serum with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent.	
Plate Sealers	640445	4 adhesive foil strips.	
Standard Value Card	749829	1 card listing the Standard Cocktail reconstitution volume and working standard concentrations for this kit lot.	

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

OTHER SUPPLIES REQUIRED

- Luminex® MAGPIX®, Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.
- Luminex® Performance Assay Control (optional; R&D Systems®, Catalog # QC18).

PRECAUTIONS

Calibrator Diluent RD6-62 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

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.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifuging 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.*

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

Use polypropylene tubes.

Note: *On the day of the assay, ALL fresh and previously frozen serum and plasma samples require centrifugation at 16,000 x g for 4 minutes immediately prior to use or dilution.*

Urine samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-62 (diluted 1:5)*. Mix thoroughly.

When assaying IP-10, Lipocalin-2, OPN, TIM-1, and TFF3, serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-62 (diluted 1:5)*. Mix thoroughly.

When assaying Clusterin, Cystatin C, and RBP4, serum and plasma samples must be diluted to a final 4000-fold dilution. A suggested 4000-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD6-62 (diluted 1:5)*. Add 25 µL of the diluted sample to 975 µL of Calibrator Diluent RD6-62 (diluted 1:5)* to complete the 4000-fold dilution. Mix thoroughly.

* See Reagent Preparation section.

REAGENT PREPARATION

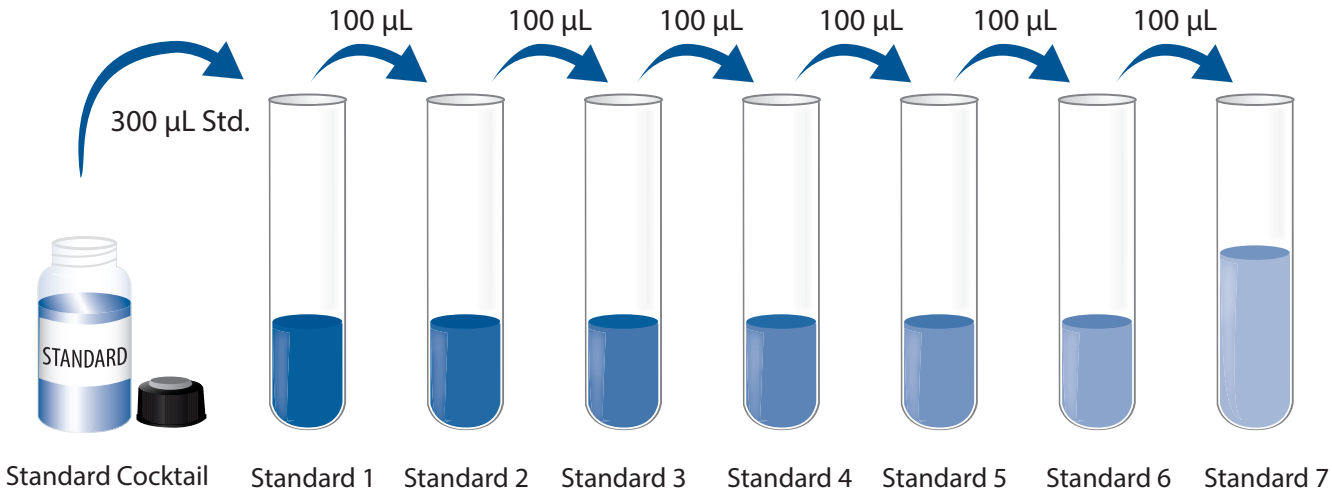
Bring all reagents to room temperature before use.

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.

Calibrator Diluent RD6-62 (diluted 1:5) - Add 20 mL of Calibrator Diluent RD6-62 to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD6-62 (diluted 1:5).

Standard - **Refer to the Standard Value Card for the reconstitution volume and assigned values.** Reconstitute the Kidney Biomarker Standard Cocktail with Calibrator Diluent RD6-62 (diluted 1:5). Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 300 µL of reconstituted Standard into a tube labeled Standard 1. Pipette 200 µL of Calibrator Diluent RD6-62 (diluted 1:5) into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD6-62 (diluted 1:5) serves as the blank.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Microparticle Diluent
96	1000 µL	+	9.5 mL
72	750 µL	+	7.125 mL
48	500 µL	+	4.75 mL
24	250 µL	+	2.375 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN-ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin-Antibody Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin-Antibody Cocktail in Diluent RD2-1. Mix gently.

Number of Wells Used	Biotin-Antibody Cocktail	+	Diluent RD2-1
96	1000 µL	+	4.5 mL
72	750 µL	+	3.375 mL
48	500 µL	+	2.25 mL
24	250 µL	+	1.125 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Streptavidin-PE concentrate in Wash Buffer.

Number of Wells Used	Streptavidin-PE Concentrate	+	Wash Buffer
96	55.0 µL	+	5.50 mL
72	42.0 µL	+	4.10 mL
48	28.0 µL	+	2.75 mL
24	14.0 µL	+	1.35 mL

INSTRUMENT SETTINGS

Note: *Adjust the probe height setting on the analyzer to avoid puncturing the plate. Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

Luminex® MAGPIX® analyzer:

a) Sample volume: 50 µL

b) Assign the microparticle region for each analyte being measured (see page 1)

c) 50 count/region

d) Collect Median Fluorescence Intensity (MFI)

Luminex® 100/200™, Luminex® FLEXMAP 3D® and Bio-Rad Bio-Plex analyzers:

Note: *Ensure that the instrument flow rate is set to the default of 60 µL/minute (fast) for all flow based analyzers.*

a) Sample volume: 50 µL

b) Bead Type:

i. Luminex® 100/200™ and FLEXMAP 3D® select MagPlex

ii. Bio-Rad Bio-Plex Manager use Bio-Plex MagPlex Beads (Magnetic)

c) Doublet Discriminator gates:

i. Luminex® 100/200™ and FLEXMAP 3D® set at 8000 and 16,500

ii. Bio-Rad Bio-Plex Manager set at 8000 and 23,000

d) Reporter Gain Setting:

i. Luminex® 100/200™ use Default setting

ii. Luminex® FLEXMAP 3D® use Standard PMT setting

iii. Bio-Rad Bio-Plex Manager use the low RP1 target value for the CAL2 setting

e) Assign the microparticle region for each analyte being measured (see page 1)

f) 50 count/region

g) Collect MFI

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 100 µL of the Microparticle Cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 1 minute before removing the liquid, filling each well with Wash Buffer (100 µL) and allow 1 minute before removing the liquid again. Complete removal of liquid is essential for good performance. **Note:** ***Do NOT blot; this may cause a loss of microparticles.*** Perform the wash procedure three times.

   Note: *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*
5. Add 50 µL of diluted Biotin-Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex® or Bio-Rad analyzer.
    Note: *Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker at 800 ± 50 rpm.*

*Samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

❶ Prepare all reagents as instructed.

↓

❷ Add 50 µL of standard, control, or sample* to each well.

↓

❸ Add 100 µL of diluted Microparticle Cocktail to each well.
Incubate for 3 hours at RT on a shaker at 800 rpm.

↓

❹ Wash by removing the liquid from each well, filling with 100 µL Wash Buffer, and removing the liquid again.
Perform the wash 3 times.

↓

❺ Add 50 µL of diluted Biotin-Antibody Cocktail to each well.
Cover and incubate for 1 hour at RT on the shaker at 800 rpm.

↓

❻ Repeat the wash as in step 4.

↓

❼ Add 50 µL of diluted Streptavidin-PE to each well.
Incubate for 30 minutes at RT on the shaker at 800 rpm.

↓

❽ Repeat the wash as in step 4.

↓

❾ Add 100 µL of Wash Buffer to each well.
Incubate for 2 minutes at RT on the shaker at 800 rpm.

↓

❿ Read within 90 minutes using a Luminex® or Bio-Rad analyzer
Note: *Resuspend microparticles immediately prior to reading.*

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

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

CALIBRATION

This assay is calibrated against highly purified recombinant human kidney biomarkers produced at R&D Systems®.

REFERENCES

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4. Goodsaid, F.M. *et al.* (2009) Clin. Pharmacol. Ther. **86**:490.
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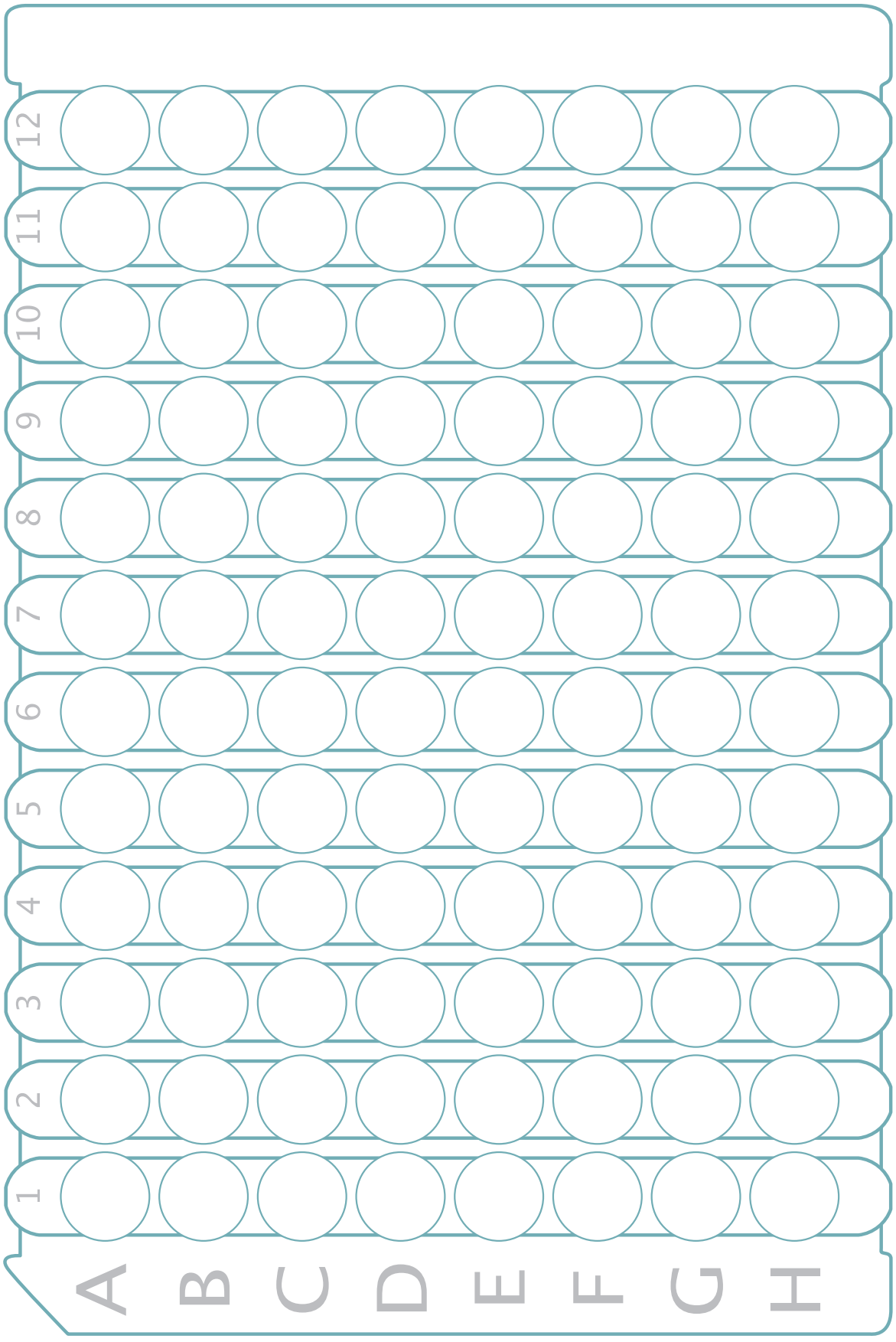
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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