Luminex® Assay

Mouse Premixed Multi-Analyte Kit

Catalog Number LXSAMSM

For the simultaneous detection of multiple mouse biomarkers in cell culture supernates, tissue lysates, 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

This kit contains the components required to screen up to 50 mouse biomarkers in cell culture supernate, tissue lysate, serum, and plasma samples in multiplexed sandwich ELISAs.

Luminex® Assays can be used to assess the levels of biomarkers of your choosing in a single sample. For ease of use, the microparticles are premixed in one vial as are the biotinylated detection antibodies.

PRINCIPLE OF THE ASSAY

The Luminex® 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 cytometry-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique bead 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 beads. One LED excites the dyes inside each bead to identify the region and the second LED excites the PE to measure the amount of analyte bound to the bead. A sample from each well is imaged with a CCD camera with a set of filters 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 bead to identify the bead region and the second laser to excite the PE to measure the amount of analyte bound to the bead. All fluorescence emissions from each bead as it passes through the flow cell is then analyzed to differentiate emission 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 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.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.
- Luminex® Assays afford the user the benefit of multi-analyte analysis of biomarkers in a single sample. A multipurpose diluent is used to dilute samples, if necessary, and provide accurate estimates of natural analytes in cell culture supernates, tissue lysates, serum, and plasma.
- **Only the analytes listed on the enclosed Certificate of Analysis can be measured with this kit.**

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 photo bleaching.

PRECAUTIONS

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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
This kit contains sufficient materials to run multiplex assays on one 96 well plate.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Mouse Standard Cocktail A†	894658	2 vials of recombinant mouse biomarkers in a buffered protein base with preservatives; lyophilized.	Use fresh standard(s) for each assay. Discard after use.
Mouse Standard Cocktail B†	894659		
Mouse Standard Cocktail C†	896020		
Mouse Standard Cocktail D†	896021		
Mouse Standard Cocktail E†	896022		
Mouse Standard Cocktail F†	896023		
Mouse Standard Cocktail G†	896024		
Mouse Standard Cocktail H†	896025		
Mouse Standard Cocktail I†	896026		
Mouse Standard Cocktail J†	896027		
Mouse Standard Cocktail K†	898612		
Mouse Magnetic Premixed Microparticle Cocktail	894724	0.6 mL of a concentrated microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Prepare fresh 1X solutions at the time of assay. Discard after use.*
Mouse Premixed Biotin-Ab Cocktail	894666	0.6 mL of a concentrated biotinylated antibody cocktail with preservatives.	
Streptavidin-PE Concentrate	893535	0.250 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6-52	895438	3 vials (21 mL/vial) 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.*	
Microplate	641385	1 flat-bottomed 96 well microplate used as a vessel for the assay.	
Certificate of Analysis	752890	1 sheet listing the selected analytes with the microparticle regions, standard reconstitution volumes, and concentrations for the provided Standard(s).	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Assay Diluent RD2-1.	
Plate Sealers	640445	4 adhesive foil strips.	

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

†Each premixed kit may contain 1 or more of the unique Standard Cocktails (A-K), depending upon the analytes selected.

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 plate washer with a magnetic platform
- Pipettes and pipette tips
- Deionized or distilled water
- Multi-channel pipette, manifold dispenser, or automated dispensing unit
- 500 mL graduated cylinder
- 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

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347)
- PBS

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.

Tissue Lysates - Brains from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Assay immediately, or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow 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.

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.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

To determine the appropriate dilution for each analyte, refer to the table located in the following link http://www.rndsystems.com/Products/LXSAMSM.

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.*

Cell culture supernate, tissue lysate, serum and plasma samples require at least a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-52. Mix thoroughly.

High abundance biomarkers may require additional dilution such as 50-,200-,4000- or 40,000-fold.

A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD6-52. Mix thoroughly.

A suggested 200-fold dilution can be achieved by adding 10 µL of sample to 90 µL of Calibrator Diluent RD6-52. Complete the 200-fold dilution by adding 10 µL of the diluted sample to 190 µL Calibrator Diluent RD6-52.

A suggested 4000-fold dilution can be achieved by adding 10 µL of 200-fold diluted sample to 190 µL of Calibrator Diluent RD6-52.

A suggested 40,000-fold dilution can be achieved by adding 20 µL of 4000-fold diluted sample to 180 µL Calibrator Diluent RD6-52.

REAGENT PREPARATION

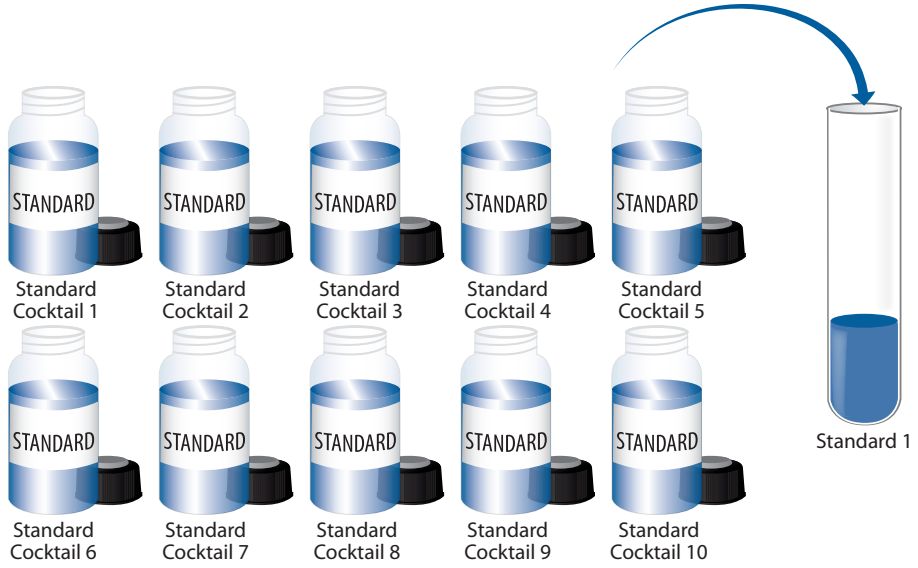
Bring all reagents to room temperature before use.

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.

Standards - Refer to the Certificate of Analysis for reconstitution volumes and assigned values. The standards provided in the kit will differ depending on the analytes selected, but may include up to 10 unique Standard Cocktails (A-K). Reconstitute 1 each of the unique Standard Cocktails provided in the kit with Calibrator Diluent RD6-52. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Upon reconstitution, each Standard Cocktail is a 10X concentrate.

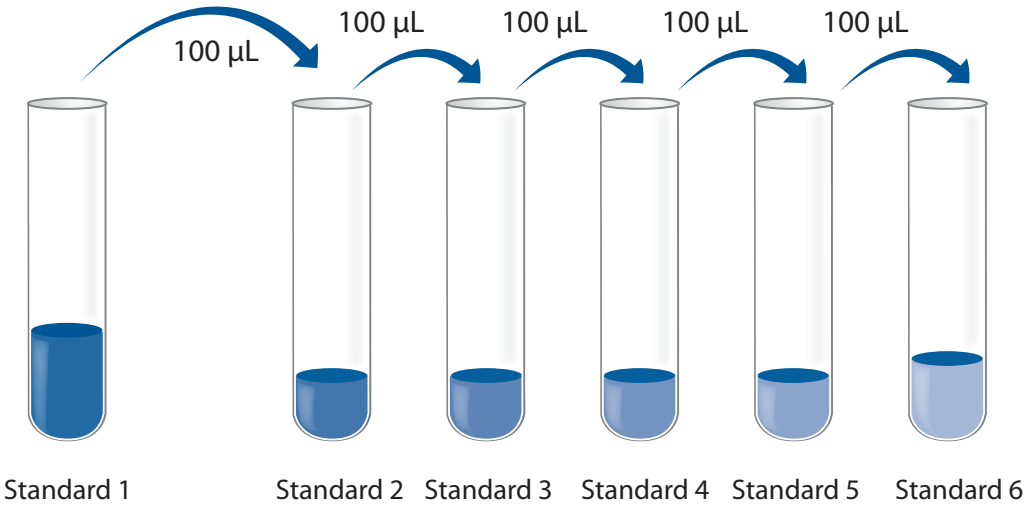
Use polypropylene tubes. Create Standard 1 by combining 100 µL of each standard cocktail with Calibrator Diluent RD6-52 into your standard 1 tube. The final volume in the standard 1 tube will be 1000 µL. Use the table below to determine how much calibrator diluent to use based on the number of standard cocktails you are combining.

Number of Unique Standard Cocktails Provided	Volume to Combine into a Single Tube	Volume of Calibrator Diluent Required	Total Volume of Standard 1
1	100 µL	900 µL	1000 µL
2	100 µL of each	800 µL	1000 µL
3	100 µL of each	700 µL	1000 µL
4	100 µL of each	600 µL	1000 µL
5	100 µL of each	500 µL	1000 µL
6	100 µL of each	400 µL	1000 µL
7	100 µL of each	300 µL	1000 µL
8	100 µL of each	200 µL	1000 µL
9	100 µL of each	100 µL	1000 µL
10	100 µL of each	0 µL	1000 µL



REAGENT PREPARATION *CONTINUED*

Pipette 200 µL of Calibrator Diluent RD6-52 into each of 5 test tubes labeled 2-6. 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 RD6-52 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 using Assay Diluent RD1W in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Assay Diluent RD1W
96	500 µL	+	5.00 mL
72	375 µL	+	3.75 mL
48	250 µL	+	2.50 mL
24	125 µL	+	1.25 mL

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

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 Assay Diluent RD1W. Mix gently.

Number of Wells Used	Biotin-Antibody Cocktail	+	Assay Diluent RD1W
96	500 µL	+	5.00 mL
72	375 µL	+	3.75 mL
48	250 µL	+	2.50 mL
24	125 µL	+	1.25 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene test tube wrapped with aluminum foil. Protect the 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	220 µL	+	5.35 mL
72	165 µL	+	4.00 mL
48	110 µL	+	2.65 mL
24	55 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:

i. 1-25 analytes: 50µL

ii. > 25 analytes: 35 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 Enhanced PMT (High) 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 Certificate of Analysis)

f) 50 count/region

g) Collect MFI

ASSAY PROCEDURE

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

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

1. Prepare all standards, reagents and samples as directed in the previous sections.
2. Add 50 µL of standard 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 50 µL of the microparticle cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 2 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 each well. 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 each well. 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 a 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 may 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 or sample* to each well.

↓

❸ Add 50 µL of diluted Microparticle Cocktail to each well.
Incubate for 2 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 may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Use the Standard concentrations on the Certificate of Analysis 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.

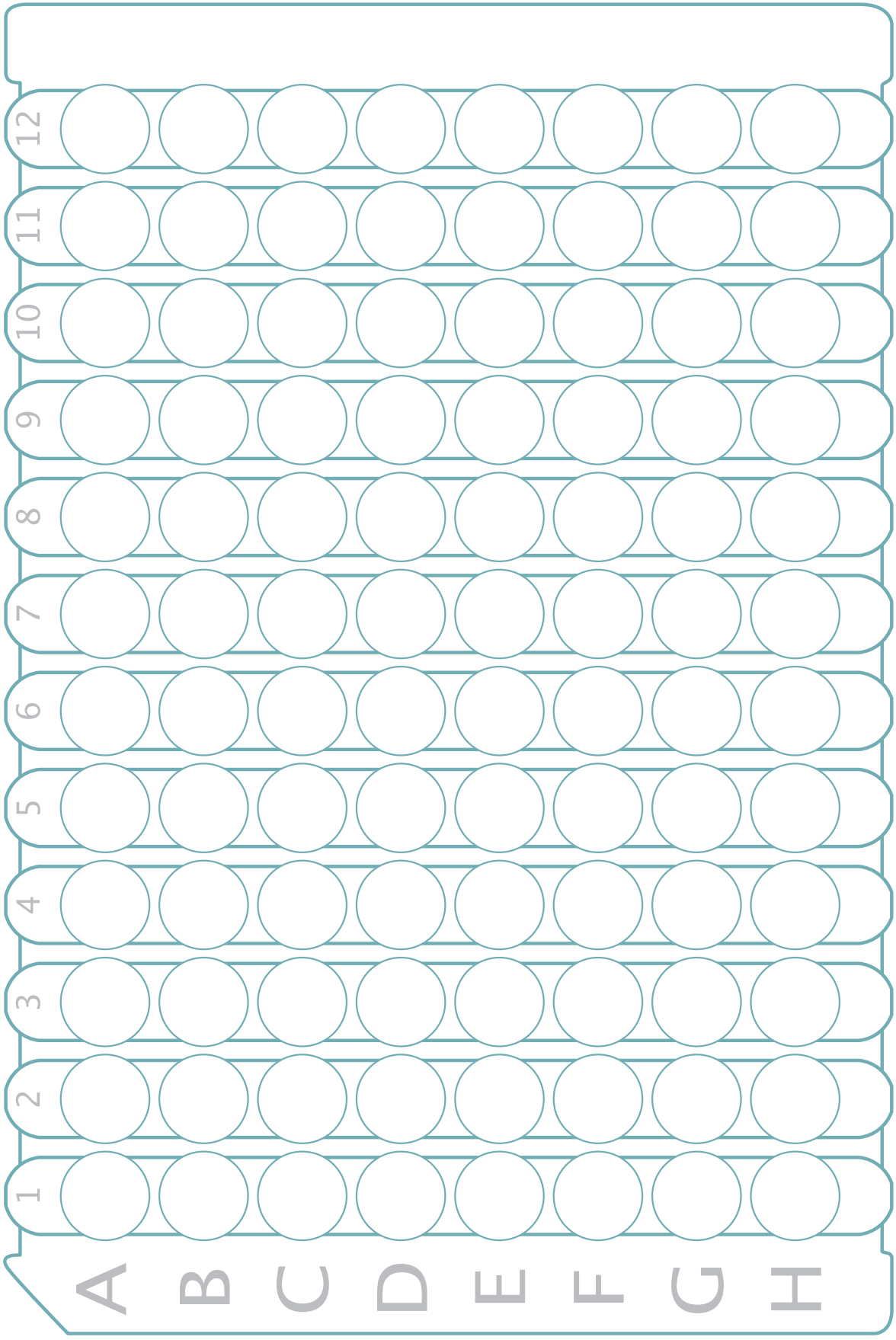
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

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

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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