Quantikine® ELISA

Mouse EGF R Immunoassay

Catalog Number MEGFR0

For the quantitative determination of mouse Epidermal Growth Factor Receptor (EGF R) concentrations in cell culture supernates, 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 Epidermal Growth Factor Receptor (EGF R), also known as Her1, ErbB1, and ErbB, is a member of the EGF R subfamily of receptor tyrosine kinases (RTKs). The EGF R family also includes ErbB2 (Neu, Her-2), ErbB3 (Her3), and ErbB4 (Her4) (1). The mature EGF R is a 170 kDa type 1 transmembrane glycoprotein that contains a 623 amino acid (aa) ligand-binding extracellular domain (ECD), a 23 aa short transmembrane domain, and a 540 aa intracellular cytoplasmic tail. The ECD is responsible for ligand binding and subsequent receptor dimerization. Within the ECD, mouse EGF R shares 88% and 93% aa sequence identity with human and rat EGF R, respectively. It shares 44-48% aa sequence identity with the ECD of mouse ErbB2, ErbB3, and ErbB4. EGF R is widely expressed on epithelial cells, predominantly in the gastrointestinal tract and breast, and is required for epithelial cell development and proliferation (2-4).

EGF R serves as the receptor for a subset of EGF family members, including EGF, TGF-α, HB-EGF, Amphiregulin, Betacellulin, Epiregulin, and Epigen (5, 6). These molecules are expressed as transmembrane proteins but are shed from the cell surface by proteolytic cleavage (7). Ligand binding induces EGF R homodimerization as well as heterodimerization with ErbB2, ErbB3, or ErbB4. Dimerization results in the activation of cytoplasmic kinase domains, tyrosine autophosphorylation, and internalization of the receptor-ligand complex (8, 9). Signaling through EGF R influences cell proliferation, motility, survival, and differentiation (1-4). Alterations in the structure, expression, and signaling of EGF R are involved in the metastasis of a wide variety of cancers, particularly those of epithelial origin (10, 11). Overexpression of the receptor is often found in advanced-stage carcinomas and correlates with a poor clinical prognosis (12, 13).

Soluble EGF R isoforms can be generated by alternative splicing or proteolytic cleavage. Soluble ECD fragments of 60kDa, 80kDa, and 110kDa retain ligand binding capability and can dimerize with membrane bound EGF R to inhibit tyrosine kinase activity (14-17). Levels EGF R have been found to be elevated in the serum of patients with skin, cervical, ovarian, breast, or colorectal cancer (18-21). Serum EGF R elevation is also associated with responsiveness to chemotherapy in advanced colorectal cancer (21). In contrast, serum EGF R is reduced in ovarian carcinoma, non-small cell lung cancer, and head and neck carcinoma (22, 23). A decrease of serum EGF R relative to soluble ErbB2 is associated with decreased life expectancy in patients with metastatic breast cancer (24). Evidence linking EGF R, tumorogenesis, and cancer progression has instigated clinical use of targeted treatments against the receptor and its signaling components (25).

The Quantikine Mouse EGF R Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse EGF R levels in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant mouse EGF R and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse EGF R accurately. Results obtained using natural mouse EGF R showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse EGF R.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse EGF R has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse EGF R present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse EGF R 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 EGF R 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, 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 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 EGF R Microplate	894740	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse EGF R.	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 EGF R Standard	894742	2 vials of recombinant mouse EGF R in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard and control for each assay.
Mouse EGF R Control	894743	2 vials of recombinant mouse EGF R in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse EGF R Conjugate	894741	12 mL of a polyclonal antibody specific for mouse EGF R conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-34	895265	11 mL of a buffer with blue dye and preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base 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.*	
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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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.

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.

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

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

Serum and plasma samples require a 500-fold dilution due to high endogenous values. A suggested 500-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5P (diluted 1:5)*. Complete the 500-fold dilution by adding 50 µL of the diluted sample to 450 µL Calibrator Diluent RD5P (diluted 1:5).

Urine samples require an 8-fold dilution due to matrix effects. A suggested 8-fold dilution is 20 µL of sample + 140 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse EGF R Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

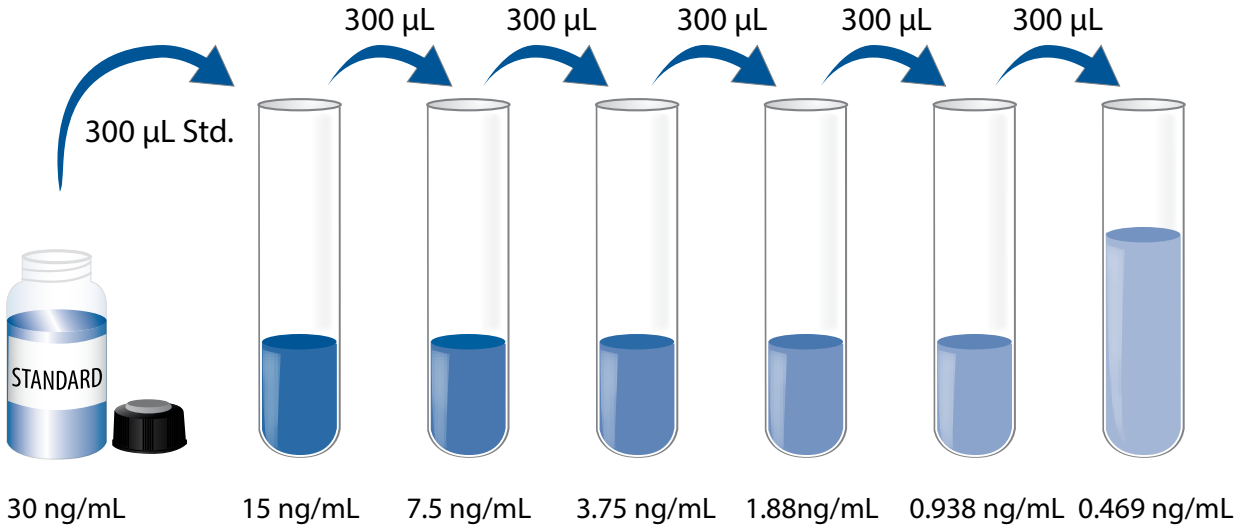
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

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 EGF R Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse EGF R Standard with Calibrator Diluent RD5P (diluted 1:5). This reconstitution produces a stock solution of 30 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse EGF R Standard (30 ng/mL) serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/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, working standards, 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 RD1-34 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. A plate layout is provided to record standards and samples assayed.

   Note: *Samples, controls, and standards 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 100 µL of Mouse EGF R Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 4.
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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

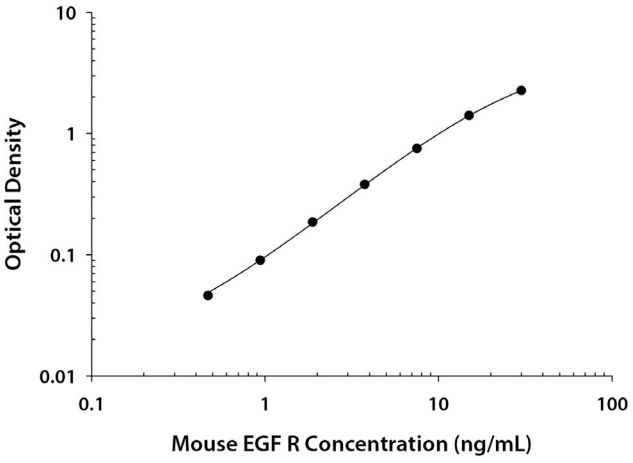
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse EGF R 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.



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.013	0.012	—
0.469	0.056 0.059	0.058	0.046
0.938	0.098 0.105	0.102	0.090
1.88	0.198 0.198	0.198	0.186
3.75	0.383 0.400	0.392	0.380
7.5	0.742 0.783	0.763	0.751
15	1.405 1.435	1.420	1.408
30	2.241 2.301	2.271	2.259

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 (ng/mL)	1.11	2.58	5.41	0.976	2.42	5.13
Standard deviation	0.049	0.080	0.159	0.090	0.114	0.197
CV (%)	4.4	3.1	2.9	9.2	4.7	3.8

RECOVERY

The recovery of mouse EGF R spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	94-107%
Urine* (n=4)	86	79-94%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse EGF R were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	97	100	102	101	105
	Range (%)	94-99	98-103	100-105	99-104	103-107
1:4	Average % of Expected	96	103	103	103	109
	Range (%)	94-98	97-107	101-106	100-106	104-112
1:8	Average % of Expected	96	108	104	104	114
	Range (%)	94-99	98-114	103-107	101-108	103-123
1:16	Average % of Expected	96	108	109	108	117
	Range (%)	94-100	97-115	105-112	102-114	104-126

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty assays were evaluated and the minimum detectable dose (MDD) of mouse EGF R ranged from 0.016-0.127 ng/mL. The mean MDD was 0.038 ng/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 NS0-derived recombinant mouse EGF R produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of EGF R in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	7118	3008-15,839	3689
EDTA plasma (n=5)	7909	3522-14,214	4934
Heparin plasma (n=5)	8514	2711-11,384	3520
Urine (n=5)	23.3	12.7-41.7	11.1

Cell Culture Supernates - Organs from mice were removed, rinsed in PBS, and kept on ice. The organs were then homogenized using a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 2 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse EGF R.

Tissue Type	(ng/mL)
Brain	0.753
Liver	9.13

SPECIFICITY

This assay recognizes natural and recombinant mouse EGF R.

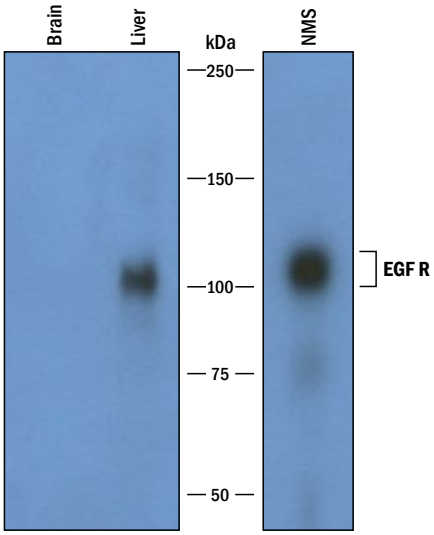
The factors listed below were prepared in 300 ng/mL of Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors in 300 ng/mL of a mid-range recombinant mouse EGF R control were assayed for interference. No significant cross-reactivity or interference was observed.

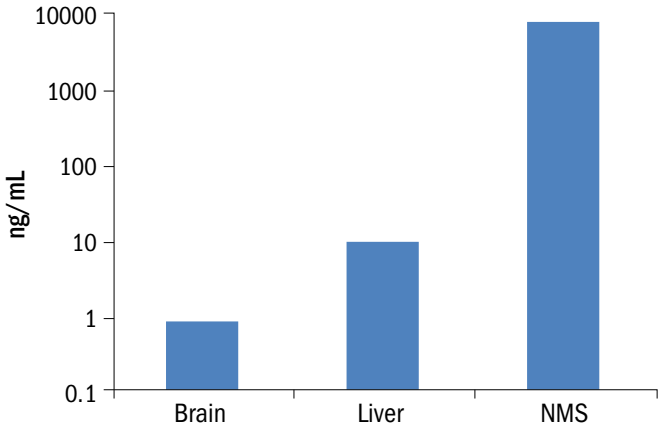
Recombinant mouse:

Amphiregulin
Betacellulin
EGF
EGF (pro)
Epigen
Epiregulin
ErbB2
ErbB3
ErbB4
HB-EGF

Recombinant human:

EGF
EGF (pro)
EGF R
Epigen
HB-EGF
TGF-α





Conditioned media from mouse brain, liver, and normal mouse serum (NMS) samples were analyzed by Western Blot and Quantikine ELISA. Conditioned media and serum samples 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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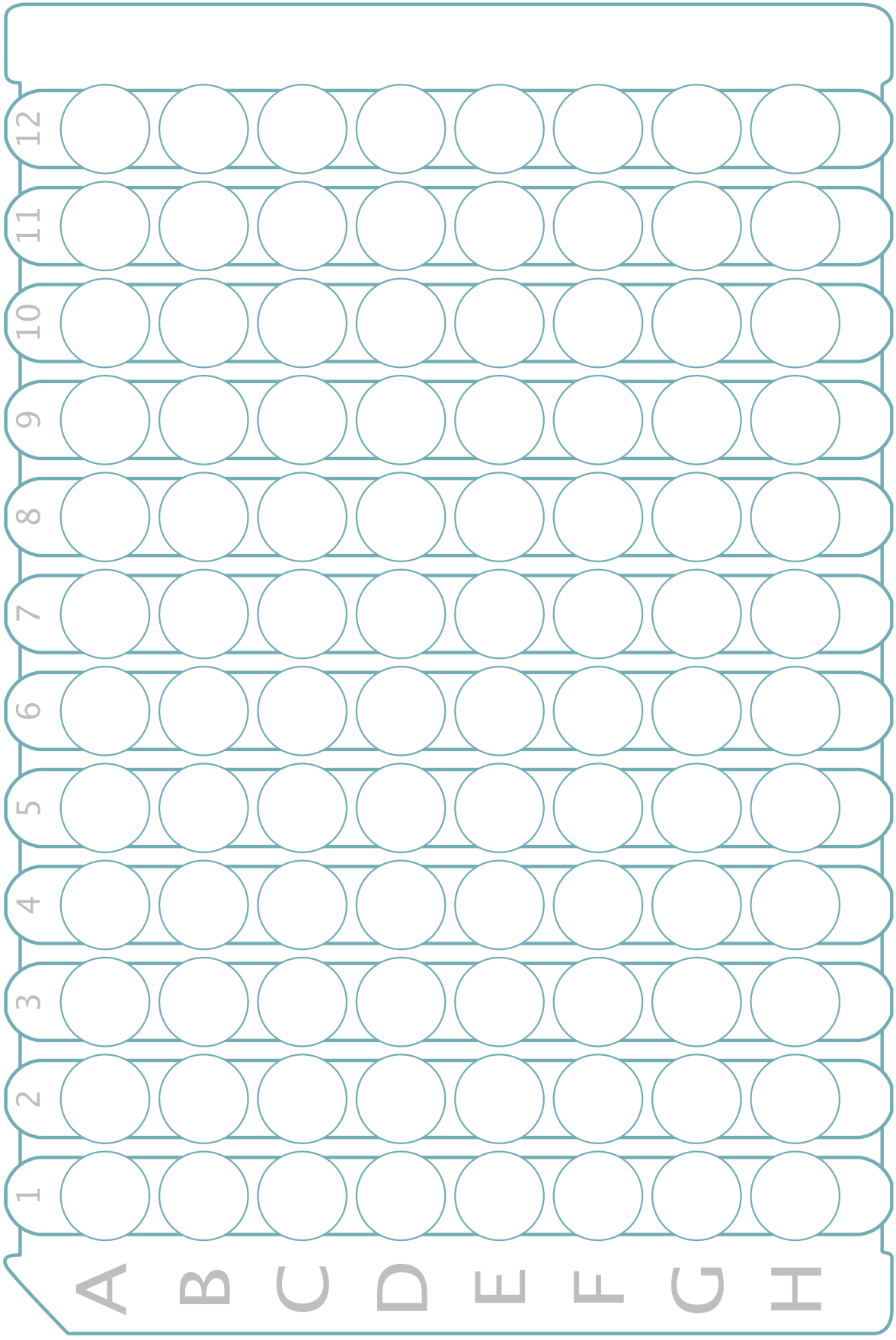
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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