Quantikine® ELISA

Mouse EGF Immunoassay

Catalog Number MEG00

For the quantitative determination of mouse Epidermal Growth Factor (EGF) concentrations in cell culture supernates, tissue homogenates, serum, plasma, and urine.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

Epidermal growth factor (EGF) is a 6 kDa non-glycosylated monomeric protein consisting of 53 amino acid (aa) residues (1-6). It is derived from a 150-170 kDa type I transmembrane pro-protein. Mouse pro-EGF precursor is synthesized as a 1217 aa protein with a 28 aa signal peptide, a 1010 aa extracellular domain, a 20 aa transmembrane region and a 159 aa cytoplasmic domain. A splice isoform of pro-EGF with a truncated cytoplasmic domain has also been reported (7). The extracellular region of mouse pro-EGF contains eight LDL-receptor class B repeats and nine EGF domains. Bioactive mature EGF is proteolytically cleaved and released only from the most membrane-proximal EGF domain (4-6, 8). A soluble 160 kDa pro-EGF containing the extracellular region of the transmembrane pro-protein can also be released proteolytically (9-11). Metalloproteases of the ADAM family, especially ADAM10, have been shown to mediate the release of pro-EGF extracellular domain and mature EGF (12). Mature and soluble pro-EGF can be detected in various body fluids and secretions including blood, urine, saliva, and milk (3). Within the active EGF domain, mouse EGF shares 70%, 73%, and 74% aa sequence identity with human, rat and porcine EGF, respectively (13). Cells known to express EGF include platelets (12), cerebral neurons (3), astrocytes, cerebellar Purkinje cells (3), cells of the Brunner (duodenum) and submandibular glands (14), non-pigmented ciliary epithelium (15), and cells of the anterior pituitary (16).

EGF signals via binding to the EGF receptor (EGF R) (also known as HER1 or ErbB1), a transmembrane receptor tyrosine kinase of the ErbB family (3, 12). Monomeric mature EGF has been shown to bind the extracellular domain of ErbB1 and induce the homodimerization with another ErbB1, or heterodimerization with ErbB2 (17). ErbB2 is an ErbB family protein that does not bind any ligands directly but is a preferred heterodimerization partner with ErbB1, 3, or 4. At high concentrations, EGF can also signal via the ErbB3:ErbB2 heterodimer (18). Cross-talk between the EGF/EGF receptor pathway with the HGF receptor pathway or the PDGF receptor β pathway has been reported (12, 19, 20). This receptor transactivation involves receptor heterodimerization and can expand the repertoire of EGF functions. In addition to mature EGF, soluble and membrane-associated pro-EGF are biologically active and are capable of binding to the EGF receptor complexes (9-11). Membrane-associated pro-EGF has been proposed to have a role in 'justacrine' cell-cell communication, and may also be capable of bidirectional signal transduction in cells on which it is expressed (17). The cytoplasmic domain of transmembrane pro-EGF has been shown to affect microtubule distribution, post-translational modification and stability (7).

A large number of diverse biological effects have been attributed to EGF. It is a mitogen that stimulates the proliferation of different types of cells, especially fibroblasts and epithelial cells (3). During development, EGF modulates growth and differentiation of thymocytes, in the passage from the double-negative to the double-positive ($CD4^+/CD8^+$) stage (21). It drives neuroglia production at the expense of neuron formation (3), promotes epithelization (22) and inhibits adipocyte maturation (22). In the adult, EGF plays a role in mammary gland lactogenesis (23). It also causes fibroblast mitosis, extracellular matrix dissociation, and migration (24).

The Quantikine Mouse EGF Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse EGF in cell culture supernates, tissue homogenates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant mouse EGF and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse EGF 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 EGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse EGF has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any EGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse EGF 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 EGF 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 the appropriate 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 Microplate	893147	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse EGF.	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 Conjugate	893148	12 mL of a polyclonal antibody conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse EGF Standard	893149	2 vials of recombinant mouse EGF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse EGF Control	893150	Recombinant mouse EGF in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *For cell culture supernate/tissue homogenate/urine samples. Use diluted 1:4 in this assay.*	
Calibrator Diluent RD6-1	895163	21 mL of diluted animal serum with preservatives. *For serum/plasma samples.*	
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.
- **Polypropylene** 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 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.

Tissue Homogenates - Prior to assay, tissues must be homogenized according to the directions in the Sample Values section. The supernate can be assayed immediately or stored 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 or lipemic samples may not be 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

Urine samples require at least 4000-fold dilution. The suggested 4000-fold dilution can be achieved by adding 10 µL of sample to 990 µL of Calibrator Diluent RD5-26 (diluted 1:4)*. Complete the 4000-fold dilution by adding 10 µL of the first solution to 390 µL of Calibrator Diluent RD5-26 (diluted 1:4).

*See Reagent Preperation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

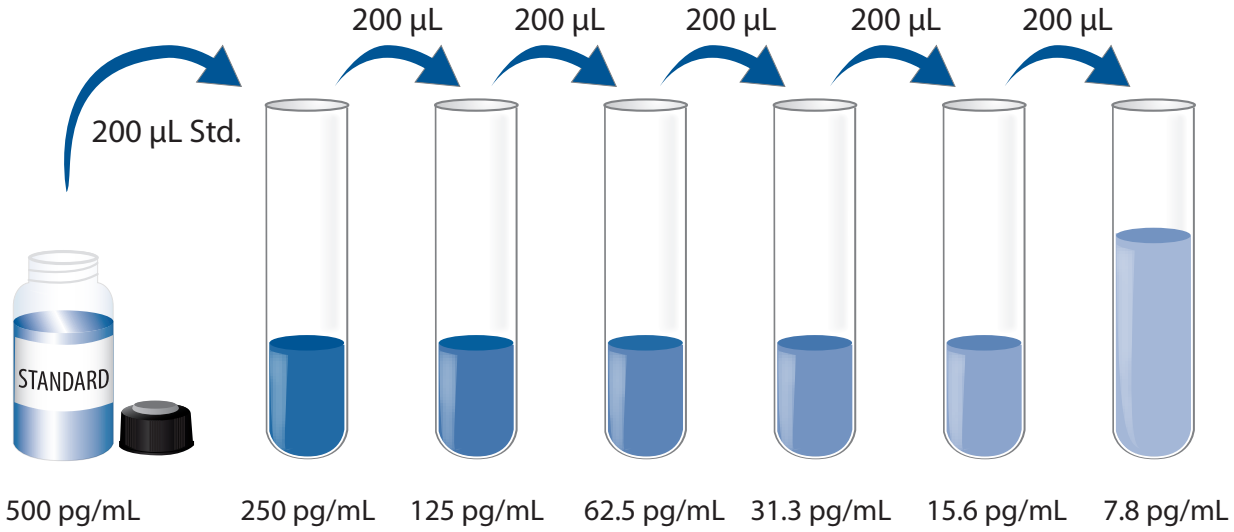
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

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 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse EGF Standard with Calibrator Diluent RD5-26 (diluted 1:4) (*for cell culture supernates/tissue homogenates/urine samples*) or Calibrator Diluent RD6-1 (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) (*for cell culture supernates/tissue homogenates/urine samples*) or Calibrator Diluent RD6-1 (*for serum/plasma samples*) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse EGF Standard (500 pg/mL) serves as the high standard. The appropriate Calibrator Diluent 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 samples, Control, and standards 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 RD1-21 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 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 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **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 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.).

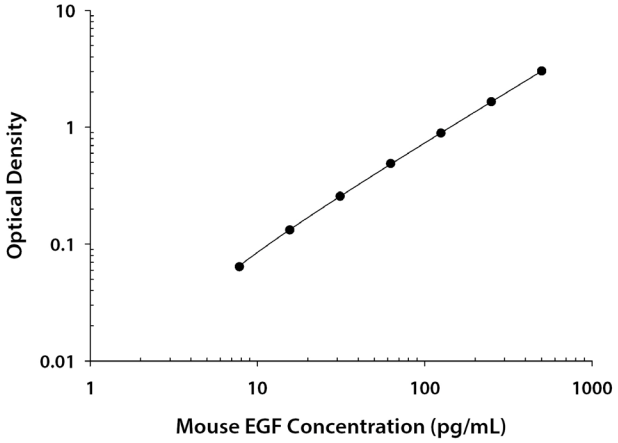
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse EGF 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 prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

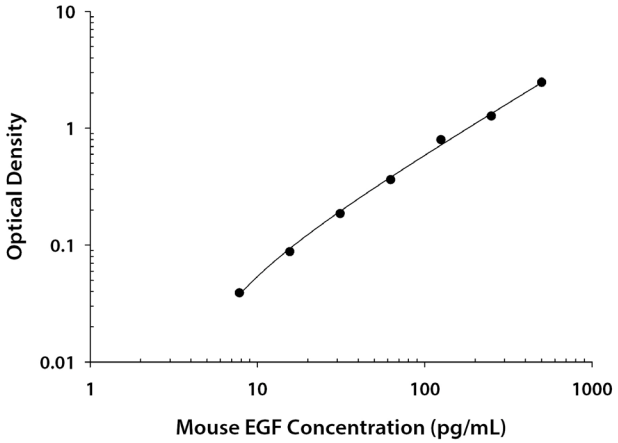
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/TISSUE HOMOGENATE/ URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.020 0.022	0.021	—
7.8	0.084 0.085	0.085	0.064
15.6	0.149 0.156	0.153	0.132
31.3	0.277 0.278	0.278	0.257
62.5	0.504 0.514	0.509	0.488
125	0.858 0.962	0.910	0.889
250	1.602 1.735	1.669	1.648
500	2.962 3.122	3.042	3.021

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.027	0.025	—
7.8	0.063 0.064	0.064	0.039
15.6	0.111 0.115	0.113	0.088
31.3	0.185 0.236	0.211	0.186
62.5	0.364 0.410	0.387	0.362
125	0.806 0.838	0.822	0.797
250	1.288 1.304	1.296	1.271
500	2.458 2.505	2.495	2.470

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 separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

CELL CULTURE SUPERNATE/TISSUE HOMOGENATE/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	27	28	27
Mean (pg/mL)	35.4	78.0	244	28.0	65.3	232
Standard deviation	3.2	7.4	11.8	2.6	4.6	18.4
CV (%)	9.0	9.5	4.8	9.3	7.0	7.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	24	25	24
Mean (pg/mL)	34.6	86.6	304	34.7	82.8	299
Standard deviation	3.4	7.9	14.7	3.2	7.7	28.4
CV (%)	9.8	9.1	4.8	9.2	9.3	9.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	109	101-119%
Tissue homogenates (n=3)	93	89-101%
Serum (n=4)	96	83-107%
EDTA plasma (n=4)	97	87-106%
Heparin plasma (n=4)	95	87-105%

SENSITIVITY

Thirty-nine assays were evaluated and the minimum detectable dose (MDD) of mouse EGF ranged from 0.32-1.64 pg/mL. The mean MDD was 0.95 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.

LINEARITY

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

		Cell culture supernates (n=5)	Tissue homogenates (n=5)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine* (n=4)
1:2	Average % of Expected	93	101	100	97	102	96
	Range (%)	92-96	95-107	92-105	92-107	96-108	86-100
1:4	Average % of Expected	93	102	99	99	98	93
	Range (%)	88-98	100-106	91-104	92-114	95-104	81-99
1:8	Average % of Expected	90	99	100	94	97	88
	Range (%)	87-93	93-109	93-109	84-113	94-104	85-91
1:16	Average % of Expected	92	98	105	94	100	88
	Range (%)	82-98	91-112	94-115	86-114	95-109	80-95

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

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse EGF produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Fifteen serum samples, twelve EDTA plasma samples, and thirteen heparin plasma samples were evaluated for detectable levels of mouse EGF in the assay. Two serum samples, one EDTA plasma sample, and one heparin plasma sample measured 8.3 pg/mL, 15.2 pg/mL, 34 pg/mL, and 12.2 pg/mL, respectively. No detectable levels were observed among the other samples.

Cell Culture Supernates - Mouse kidneys (chopped into 1-2 mm pieces) were cultured for 24 hours in 50 mL of RPMI supplemented with 10% fetal calf serum, 10 ng/mL of recombinant human IL-2, and 50 µM of 2β-mercaptoethanol. An aliquot of the cell culture supernate was removed, assayed for mouse EGF, and measured 1135 pg/mL.

Tissue Homogenates - Mouse kidneys from 4 mice were rinsed with PBS, chopped into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. Sample was frozen and thawed twice. Debris was then removed by centrifugation. The supernate was removed, assayed for mouse EGF, and measured 1220 pg/mL.

Urine - Six samples were evaluated for detectable levels of mouse EGF in this assay and measured from 595-1326 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse EGF.

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

Recombinant mouse:

Amphiregulin
Betacellulin
EGF R
Epigen
FGF-8b
FGF-8c
HGF
IGF-I
IGF-II
IGFBP-2
IGFBP-3
IGFBP-5
IGFBP-6
NGF R
PDGF-C
PDGF Rβ
PlGF-2
RAGE
TGF-β R2
VEGF
VEGF-D
VEGF R1
VEGF R2
VEGF R3
VLDLR

Recombinant rat:

EGF
PDGF-AA
PDGF-AB
PDGF-BB

Recombinant human:

EGF
TGF-α

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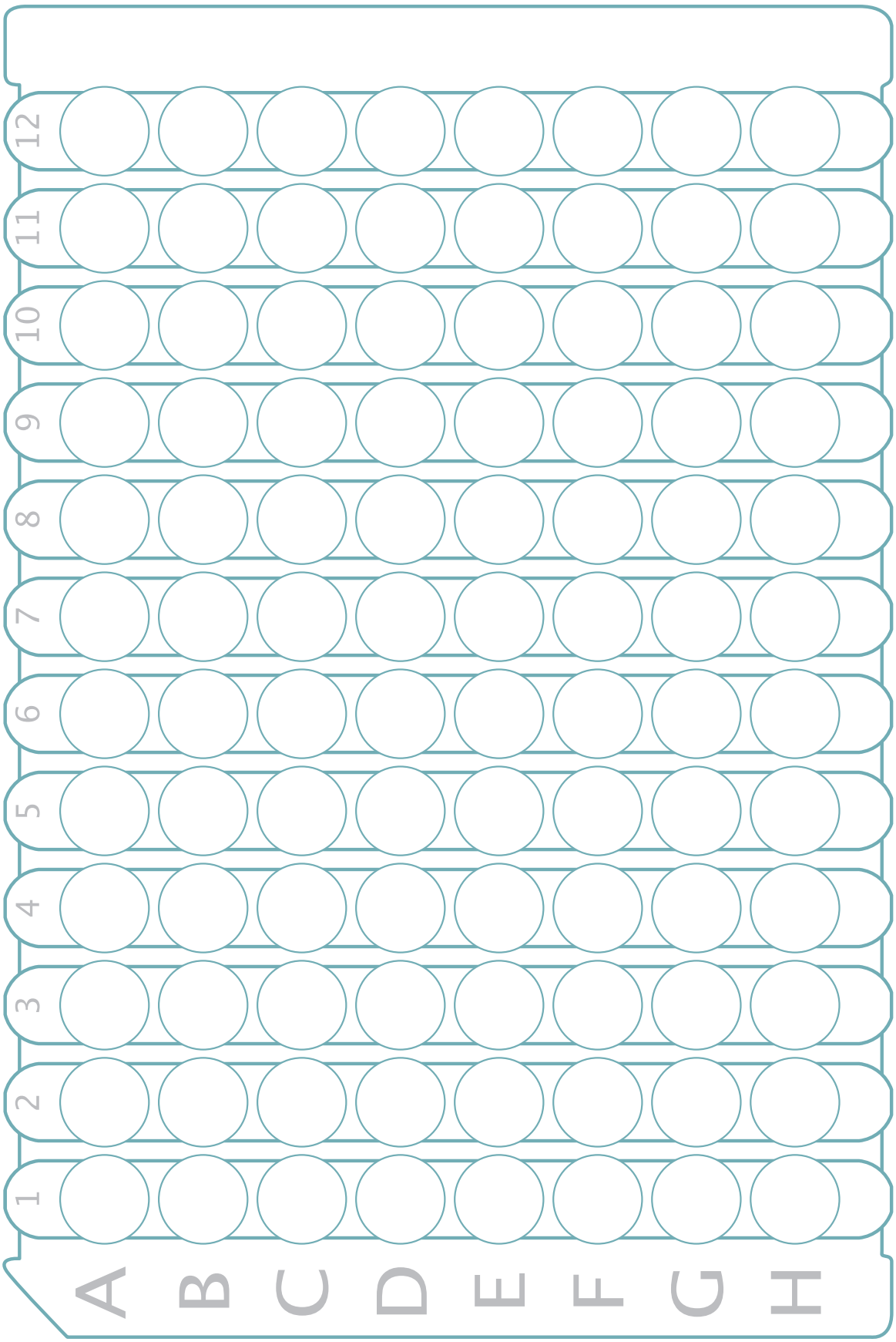
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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