Quantikine® ELISA

Mouse/Rat IGF-I Immunoassay

Catalog Number MG100
SMG100
PMG100

For the quantitative determination of mouse/rat Insulin-like Growth Factor I (IGF-I) concentrations in cell culture supernates, tissue homogenates, 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

Insulin-like Growth Factor I (IGF-I), also known as somatomedin C, is a member of the insulin superfamily (1, 2). It was originally discovered as a mediator of growth hormone actions on somatic cell growth, but has also been shown to be an important regulator of cell metabolism, differentiation and survival. IGF-I is synthesized as a preproprotein that is proteolytically cleaved to generate the mature protein linked by three disulfide bonds. Mature IGF-I is highly conserved among large mammals, with 100% sequence identity between the human, bovine, porcine, equine, and canine proteins (1).

Mature mouse IGF-I is a non-glycosylated, 70 amino acid (aa) secreted polypeptide that is derived from either a 153 aa or a 159 aa preproprotein (3). It shares 99% and 94% aa sequence identity with rat and human IGF-I, respectively. IGF-I is synthesized in the liver and other tissues. It is found in blood and other body fluids as a complex with specific high affinity IGF binding proteins (IGFBP-1 to -6) (4-6). The IGFBPs are expressed in specific patterns during development. They are modulators of IGF actions, which control IGF bioavailability to specific cell-surface receptors. Their functions are further regulated by IGFBP proteases, which proteolytically cleave the IGFBPs to lower the affinity with which they bind IGFs and increase IGF bioavailability. Some IGFBPs also have IGF-independent effects on cell functions. IGF-I circulates primarily as a ternary complex with IGFBP-3 or IGFBP-5 and the acid-labile subunit (ALS). Some IGF-I is also present in binary complexes with other IGFBPs. Whereas the ternary complexes are generally restricted to the vasculature, the binary complexes freely enter the tissues (4-6).

IGF-I actions are mediated by two type I transmembrane receptor tyrosine kinases: the IGF-I receptor (IGF-I R), and the insulin receptor (INS R) that exists in two alternatively spliced isoforms (INS R-A and -B). Both IGF-I R and INS R share a highly homologous structure and are ubiquitously expressed. Each receptor is derived from a precursor that is proteolytically cleaved into two disulfide-linked subunits: the extracellular a- and the transmembrane b-subunits. Functional IGF-I receptors are tetrameric glycoproteins composed of two disulfide-linked IGF-I Rs or disulfide-linked hybrids of one IGF-I R and one INS R. Whereas IGF-I binds with high affinity to homodimeric IGF-I R and heterodimeric IGF-I R:INS R-A or -B hybrids, high-affinity binding of insulin is observed only with dimeric INS R or IGF-I R:INS R-A hybrid but not with IGF-I R:INS R-B hybrid. The signaling responses from the various receptors are different depending whether insulin or IGF-I is used as the activating ligand (7-10).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat IGF-I has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse or rat IGF-I present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat IGF-I 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 or rat IGF-I 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, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in 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.
- 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 #	CATALOG # MG100	CATALOG # SMG100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat IGF-I Microplate	892743	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat IGF-I.	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/Rat IGF-I Standard	892745	2 vials	9 vials	4 ng/vial of recombinant mouse IGF-I in a buffered protein base with preservatives; lyophilized.	Use a new Standard and Control for each assay. Discard after use.
Mouse/Rat IGF-I Control	892746	2 vials	9 vials	Recombinant mouse IGF-I in a buffered protein base with preservatives; lyophilized. The concentration range of IGF-I after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Mouse/Rat IGF-I Conjugate	892744	1 vial	6 vials	12 mL/vial of a polyclonal antibody against mouse/rat IGF-I conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD5-38	895555	3 vials	18 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

MG100 contains sufficient materials to run an ELISA on one 96 well plate.
SMG100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMG100). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, heart and lung tissue from eight mice or one rat was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed. Alternatively, 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.*

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution into Calibrator Diluent RD5-38. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-38.

Mouse serum and plasma samples require a 500-fold dilution into Calibrator Diluent RD5-38. A 500-fold dilution can be achieved by first adding 10 µL of sample to 490 µL of Calibrator Diluent RD5-38. Complete the 500-fold dilution by adding 15 µL of this solution to 135 µL of Calibrator Diluent RD5-38.

Rat serum and plasma samples require a 1000-fold dilution into Calibrator Diluent RD5-38. A 1000-fold dilution can be achieved by first adding 10 µL of sample to 490 µL of Calibrator Diluent RD5-38. Complete the 1000-fold dilution by adding 15 µL of this solution to 285 µL of Calibrator Diluent RD5-38.

Tissue homogenates may require a 4 to 8-fold dilution.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

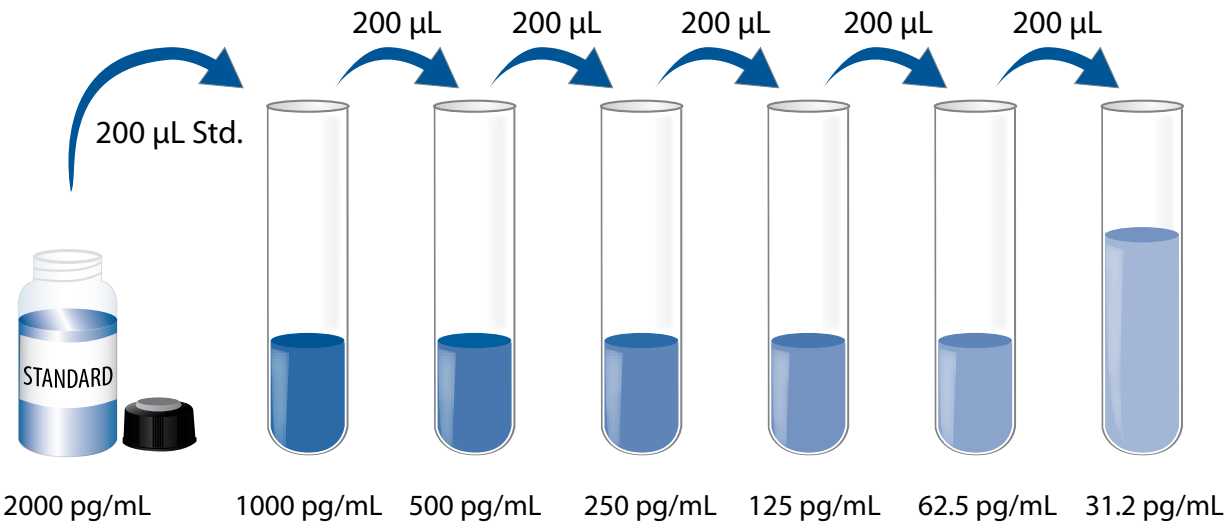
Mouse/Rat IGF-I Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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. 100 µL of the resultant mixture is required per well.

Mouse/Rat IGF-I Standard - Reconstitute the Standard with 2.0 mL of Calibrator Diluent RD5-38. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-38 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 Standard serves as the high standard (2000 pg/mL). Calibrator Diluent RD5-38 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, standards, and control 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 Calibrator Diluent RD5-38 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 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 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 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 require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

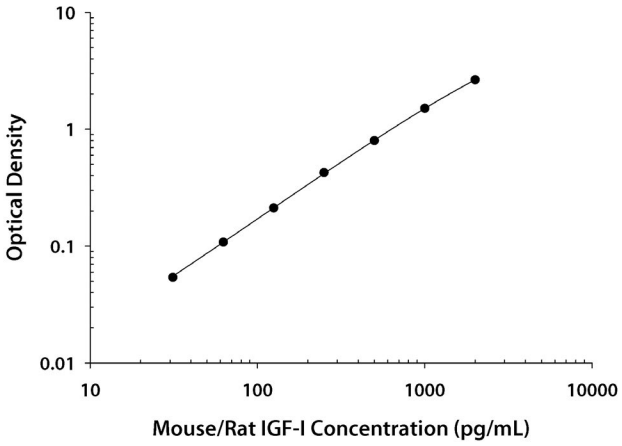
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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/rat IGF-I 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 the assay, their measured concentrations 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	0.032 0.035	0.034	—
31.2	0.087 0.089	0.088	0.054
62.5	0.139 0.144	0.142	0.108
125	0.243 0.248	0.246	0.212
250	0.449 0.469	0.459	0.425
500	0.805 0.859	0.832	0.798
1000	1.492 1.594	1.543	1.509
2000	2.606 2.747	2.677	2.643

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 thirty-five separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	35	35	35
Mean (pg/mL)	82	269	921	86	256	863
Standard deviation	4.6	8.9	37.7	7.8	11.8	37.4
CV (%)	5.6	3.3	4.1	9.1	4.6	4.3

RECOVERY

The recovery of mouse/rat IGF-I spiked to three levels throughout the range of the assay in the cell culture supernate samples was evaluated. Samples were spiked then diluted prior to assay as directed in Sample Preparation.

Sample Type	Average % Recovery	Range
Mouse cell culture supernates (n=7)	98	89-111%
Rat cell culture supernates (n=6)	98	82-108%

SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of mouse/rat IGF-I ranged from 1.6-8.4 pg/mL. The mean MDD was 3.5 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 mouse or rat IGF-I in each matrix were diluted with Calibrator Diluent RD5-38 and then assayed.

		Mouse Samples				
		Cell culture supernates* (n=3)	Tissue homogenates* (n=4)	Serum** (n=8)	EDTA plasma** (n=8)	Heparin plasma** (n=8)
1:2	Average % of Expected	100	102	96	99	97
	Range (%)	97-104	89-109	86-102	94-105	92-105
1:4	Average % of Expected	107	104	94	104	102
	Range (%)	105-109	92-111	84-103	99-111	94-112
1:8	Average % of Expected	106	106	96	105	104
	Range (%)	105-106	89-115	86-109	95-119	96-116
1:16	Average % of Expected	110	106	96	107	103
	Range (%)	109-112	81-120	84-101	98-117	94-120

		Rat Samples				
		Cell culture supernates* (n=3)	Tissue homogenates* (n=5)	Serum** (n=7)	EDTA plasma** (n=8)	Heparin plasma** (n=7)
1:2	Average % of Expected	109	96	99	98	97
	Range (%)	107-111	91-101	93-106	91-106	90-103
1:4	Average % of Expected	112	97	96	100	95
	Range (%)	106-119	90-102	90-101	89-110	87-104
1:8	Average % of Expected	114	97	97	101	101
	Range (%)	111-119	90-103	84-110	85-116	94-111
1:16	Average % of Expected	113	98	103	107	101
	Range (%)	110-117	82-109	96-112	90-118	86-117

*Mouse and rat cell culture supernates and tissue homogenate samples were diluted 2 to 8-fold, dependent on sample's value.

**Mouse and rat serum and plasma samples were diluted as directed by the Sample Preparation section.

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Individual mouse and rat serum and plasma samples were evaluated for detectable levels of mouse and rat IGF-I in this assay. Serum and plasma samples were not matched.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum* (n=20)	742	513-924	113
Mouse EDTA plasma* (n=20)	593	346-806	130
Mouse heparin plasma* (n=20)	821	542-1447	203

*Samples were diluted 500-fold prior to assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Rat serum* (n=20)	1158	436-1908	376
Rat EDTA plasma* (n=20)	1016	430-2207	377
Rat heparin plasma* (n=20)	1012	723-1444	208

*Samples were diluted 1000-fold prior to assay.

Cell Culture Supernates:
J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM containing 10% fetal bovine serum and stimulated with 2.5 ng/mL LPS. The cell culture supernate was assayed for levels of mouse IGF-I and measured 5842 pg/mL.

WEHI-3 mouse myelomonocytic leukemia cells were cultured for 2-4 days in RPMI supplemented with 10% fetal bovine serum. The cell culture supernate was assayed for levels of mouse IGF-I and measured 4944 pg/mL.

Rat lung conditioned media (1 lung, 1-2 mm pieces) was cultured for 2 days in 50 mL of DMEM supplemented with 10% fetal bovine serum and stimulated with 100 ng/mL LPS and 50 ng/mL PMA. The cell culture supernate was assayed for levels of rat IGF-I and measured 1514 pg/mL.

Rat heart conditioned media (1 heart, 1-2 mm pieces) was cultured for 4 days in 30 mL of RPMI, supplemented with 10% fetal bovine serum, and stimulated with 2.5 ng/mL LPS. The cell culture supernate was assayed for levels of rat IGF-I and measured 2888 pg/mL.

Tissue Homogenates:
Homogenates from mouse lung and mouse heart tissue were assayed for levels of mouse IGF-I and measured 5862 pg/mL and 3158 pg/mL, respectively.

Homogenates from rat lung and rat heart tissue were assayed for levels of rat IGF-I and measured 5476 pg/mL and 1785 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat IGF-I.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-38 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat IGF-I control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

EGF
EGF R
FGF-8b
FGF-8c
IGF-II
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-5
IGFBP-6
NGF R
PDGF-C

Recombinant rat:

PDGF-AA
PDGF-AB
PDGF-BB

Recombinant human:

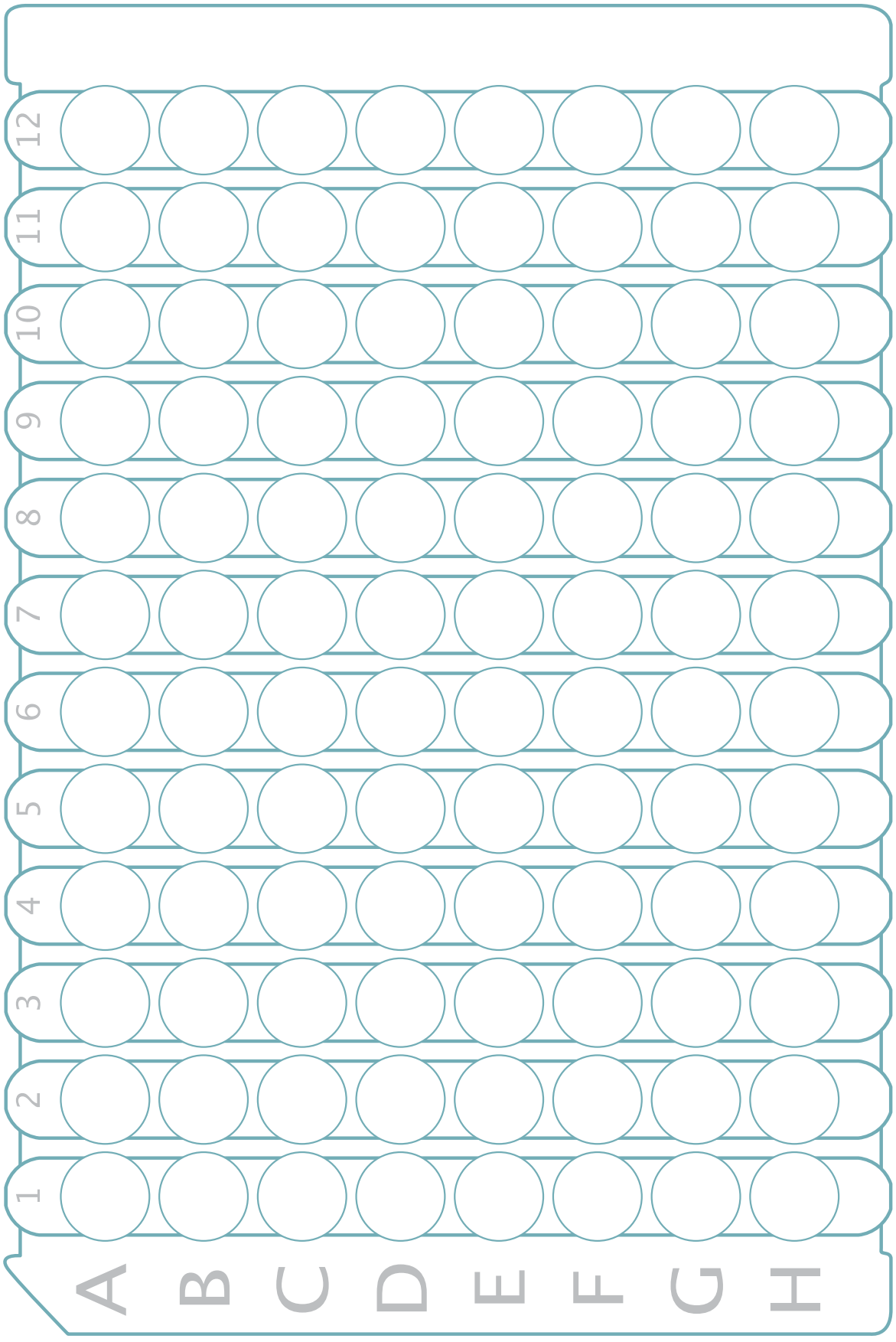
IGF-I
IGF-I sR
IGF-II
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6

REFERENCES

1. Rotwein, P. (1999) in *Molecular Biology of IGF-I and IGF-II*. Rosenfeld, R. and C.J. Roberts, eds. Humana Press, Totowa, N.J., p. 19.
2. Humbel, R.E. (1990) Eur. J. Biochem. **190**:445.
3. Grimberg, A. and P. Cohen (2000) J. Cell. Physiol. **183**:1.
4. Hwa, V. *et al.* (1999) Endocr. Rev. **20**:761.
5. Le Roith, D. *et al.* (2001) Endocr. Rev. **22**:53.
6. Boisclair, Y.R. *et al.* (2001) J. Endocrinol. **170**:63.
7. Pandini, G. *et al.* (2002) J. Biol. Chem. **277**:39684.
8. De Meyts, P. *et al.* (2004) Novartis Found. Symp. **262**:160.
9. Entingh-Pearsall, A. and C.R. Kahn (2004) J. Biol. Chem. **279**:38016.
10. Nitert, M.D. *et al.* (2005) Mol. Cell. Endocrinol. **229**:31.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES