Quantikine® ELISA

Human IGF-I Immunoassay

Catalog Number DG100
SG100
PDG100

For the quantitative determination of human Insulin-like Growth Factor I (IGF-I) concentrations in cell culture supernates, serum, and plasma.

Note: Beginning May 2013, 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

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

IGF-I (previously called somatomedin C) is a 7.6 kDa, 70 amino acid (aa) polypeptide with three internal disulfide bonds. The sequence of human IGF-I is identical to that of bovine and porcine IGF-I, and it is 70% identical to human IGF-II. IGF-I is a single-chain molecule with about 50% identity to the sequences of the A- and B-chains of human insulin. For reviews of IGF-I see references 1-3.

While IGF-I has very complex functions, it appears to largely mediate the actions of growth hormone. Thus, IGF-I is important in prenatal development, growth to adulthood, and metabolic control. It induces amino acid uptake, protein synthesis, and glucose utilization. It is an important mitogen and regulator of the cell cycle and apoptosis. IGF-I is produced primarily by hepatocytes, serving an endocrine function. It is also produced by many other cells, where it may act in an autocrine or paracrine manner. Serum levels of IGF-I have been reported to increase from birth to puberty, followed by a slow decline through adulthood (4).

There are two receptors for the IGFs; type I IGF receptor, which signals through a tyrosine kinase, and type II IGF receptor, which is identical to the mannose-6-phosphate receptor and may not signal. The action of IGF on its receptors is very complex, with control by at least six IGF-binding proteins (IGFBP-1 through IGFBP-6). IGFBP-3 binds over 90% of the total IGF in serum in a complex of IGF, IGFBP, and an acid-labile subunit. This ternary complex greatly stabilizes IGF in the circulation, changing the half-life from minutes to hours. In addition, IGFBP's modulate the action of IGF on the membrane receptors. Adding to the complexity is a family of proteases that act on IGFBPs, modifying their affinity for IGF or completely eliminating the IGFBPs. The interactions of IGF, IGFBP, IGFBP proteases, and IGF receptors are referred to as the IGF axis. The IGF axis affects many primary physiological and pathological processes, including development, growth, metabolic regulation, tumorigenesis, atherosclerosis, and angiogenesis.

The Quantikine Human IGF-I Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human IGF-I in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IGF-I and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human 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 IGF-I.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IGF-I has been pre-coated onto a microplate. Standards and pretreated samples are pipetted into the wells and any IGF-I present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for 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 and color develops in proportion to the amount of IGF-I bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the cell culture supernates or pretreated serum or plasma 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 soluble receptors, binding proteins, and 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DG100	CATALOG # SG100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
IGF-I Microplate	890773	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against 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.*
IGF-I Conjugate	890774	1 vial	6 vials	21 mL/vial of polyclonal antibody against IGF-I conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
IGF-I Standard	890775	1 vial	6 vials	Recombinant human IGF-I in a buffer with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	
Assay Diluent RD1-53	895318	1 vial	6 vials	21 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-22	895317	1 vial	6 vials	11 mL/vial of a buffer with blue dye and 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	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Pretreatment A	895319	1 vial	6 vials	21 mL/vial of an acidic dissociation solution.	
Pretreatment B	895320	1 vial	6 vials	Buffered protein with blue dye and preservatives; lyophilized.	Aliquot and store at ≤ -70 °C for up to 1 month.* Avoid repeated freeze-thaw cycles.
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DG100 contains sufficient materials to run an ELISA on one 96 well plate.
SG100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDG100). 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.
- 2-8 °C refrigerator.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human IGF-I Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution and Pretreatment A provided with this kit are acid solutions.

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.

Note: *Since bovine and porcine IGF-I share 100% sequence identity with human IGF-I, it may be necessary to run the medium alone as a control and subtract the result from the samples of conditioned medium.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at 2-8 °C for up to 4 weeks or freeze at ≤ -20 °C.

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 2-8 °C for up to 4 weeks or freeze at ≤ -20 °C.

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

SAMPLE PRETREATMENT

Serum and plasma samples must be pretreated to release IGF-I from binding proteins. Bring Pretreatment A and B to room temperature before use. Do not pretreat cell culture supernate samples, standards, or controls.

1. Add 20 µL of serum or plasma to 380 µL of Pretreatment A in a polypropylene tube.
2. Vortex and incubate for 10 minutes at room temperature.
3. Add 50 µL of sample from step 2 to 200 µL of reconstituted Pretreatment B in a polypropylene tube. Mix well. Assay immediately.

Note: *Since the samples are pretreated, the concentration read from the standard curve must be multiplied by the dilution factor, 100.*

REAGENT PREPARATION

Bring all reagents except conjugate to room temperature before use.

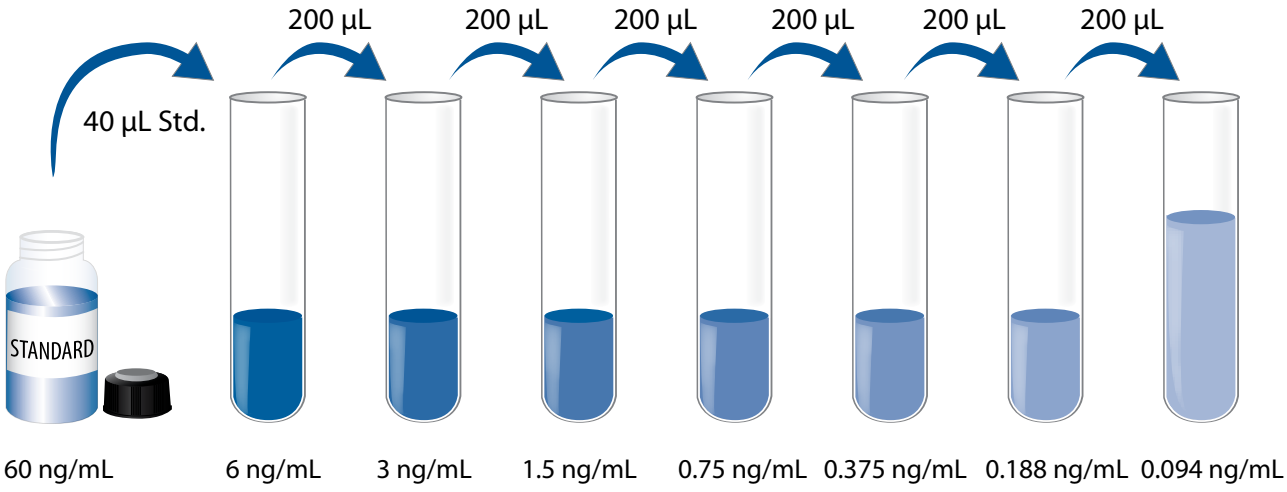
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Pretreatment B - Reconstitute Pretreatment B with 10 mL of deionized or distilled water. Allow the Pretreatment B to sit for 10 minutes with gentle agitation before using.

IGF-I Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the IGF-I Standard with deionized or distilled water. This reconstitution produces a stock solution of 60 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 360 µL of Calibrator Diluent RD5-22 into the 6 ng/mL tube. Pipette 200 µL of Calibrator Diluent RD5-22 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 6 ng/mL standard serves as the high standard. Calibrator Diluent RD5-22 serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Conjugate should remain at 2-8 °C until use.

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

1. Prepare all reagents, working standards, 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 150 µL of Assay Diluent RD1-53 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 2-8 °C.** A plate layout is provided to record standards and samples assayed.
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 200 µL of **cold** IGF-I Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour **at 2-8 °C.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes **at room temperature. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, 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.

*Serum and plasma samples require pretreatment. See Sample Pretreatment section.

CALCULATION OF RESULTS

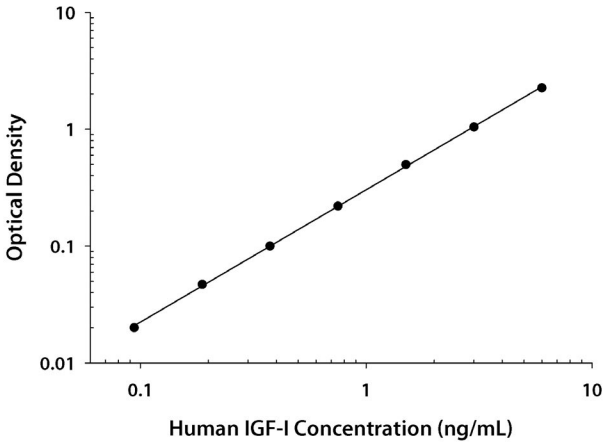
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the IGF-I concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been pretreated, the concentration read from the standard curve must be multiplied by the dilution factor, 100.

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	0.020 0.020	0.020	—
0.094	0.039 0.042	0.040	0.020
0.188	0.070 0.064	0.067	0.047
0.375	0.115 0.124	0.120	0.100
0.75	0.237 0.244	0.240	0.220
1.5	0.507 0.527	0.517	0.497
3.0	1.040 1.085	1.062	1.042
6.0	2.230 2.319	2.274	2.254

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 forty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.490	1.243	2.373	0.382	0.967	1.941
Standard deviation	0.017	0.054	0.103	0.031	0.080	0.145
CV (%)	3.5	4.3	4.3	8.1	8.3	7.5

RECOVERY

The recovery of IGF-I spiked to three different levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	97-102%

LINEARITY

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

		Cell culture media (n=4)	Cell culture supernates (n=4)	Serum* (n=6)	EDTA plasma* (n=6)	Heparin plasma* (n=6)
1:2	Average % of Expected	100	99	98	100	102
	Range (%)	98-101	92-108	90-109	94-109	94-107
1:4	Average % of Expected	97	102	97	97	103
	Range (%)	94-100	92-108	86-104	89-103	91-112
1:8	Average % of Expected	92	107	100	98	106
	Range (%)	89-94	88-114	86-106	86-115	91-115
1:16	Average % of Expected	91	———	89	97	102
	Range (%)	89-93	———	87-92	92-103	98-108

*Samples were pretreated prior to dilution.

SENSITIVITY

Thirty-four assays were evaluated and the minimum detectable dose (MDD) of IGF-I ranged from 0.007-0.056 ng/mL. The mean MDD was 0.026 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 *E. coli*-expressed recombinant human IGF-I produced at R&D Systems.

The NIBSC/WHO IGF-I International Reference Reagent 02/254 was evaluated in this kit. The dose response curve of the International Reference Reagent parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human IGF-I kit to approximate NIBSC/WHO 02/254 values, use the equation below.

NIBSC/WHO (02/254) approximate value (ng/mL) = 1.54 x Quantikine IGF-I value (ng/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of IGF-I in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=61)	105	40-258
EDTA plasma (n=35)	86	40-174
Heparin plasma (n=35)	96	43-182

Cell Culture Supernates:
Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum and 50 µM β-mercaptoethanol. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed and assayed for levels of natural IGF-I.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	0.542	1.591
Stimulated	0.447	1.309

U2OS human osteosarcoma cells were grown to 100% confluency in McCoy's 5a, 15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed and tested for levels of natural human IGF-I. The sample measured 2.192 ng/mL.

THP-1 human acute monocytic leukemia cells were seeded at 5 x 10^6 cells/mL and grown in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 µg/mL streptomycin sulfate, and 100 U/mL penicillin at 37 °C and 5% CO_2. Cells were stimulated with 1 µg/mL rhIFN-γ for 8 hours, and then 1 µg/mL LPS was added and incubated for an additional 8 hours. An aliquot of the cell culture supernate was removed and tested for levels of natural human IGF-I. The sample measured 0.596 ng/mL.

HepG2 human hepatocellular carcinoma cells (5 x 10^6 cells/mL) were grown in MEM supplemented with 5% FBS and L-glutamine. After 5 days of culture, an aliquot of the cell culture supernate was removed and tested for levels of natural human IGF-I. The sample measured 0.295 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IGF-I.

The factors listed below were prepared at 60 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 60 ng/mL in a mid-range recombinant human IGF-I standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

FGF acidic
FGF basic
HGF
IGF-II
Insulin
PDGF-AA
PDGF-AB
PDGF-BB
TGF-β1
TGF-β2
VEGF

Recombinant mouse:

IGF-I
IGF-II

Recombinant rat:

IGF-I

Serum samples were spiked with 500 ng/mL of the following binding proteins, pretreated in accordance with the protocol provided on page 5, and assayed for interference. No significant interference was observed.

Recombinant human:

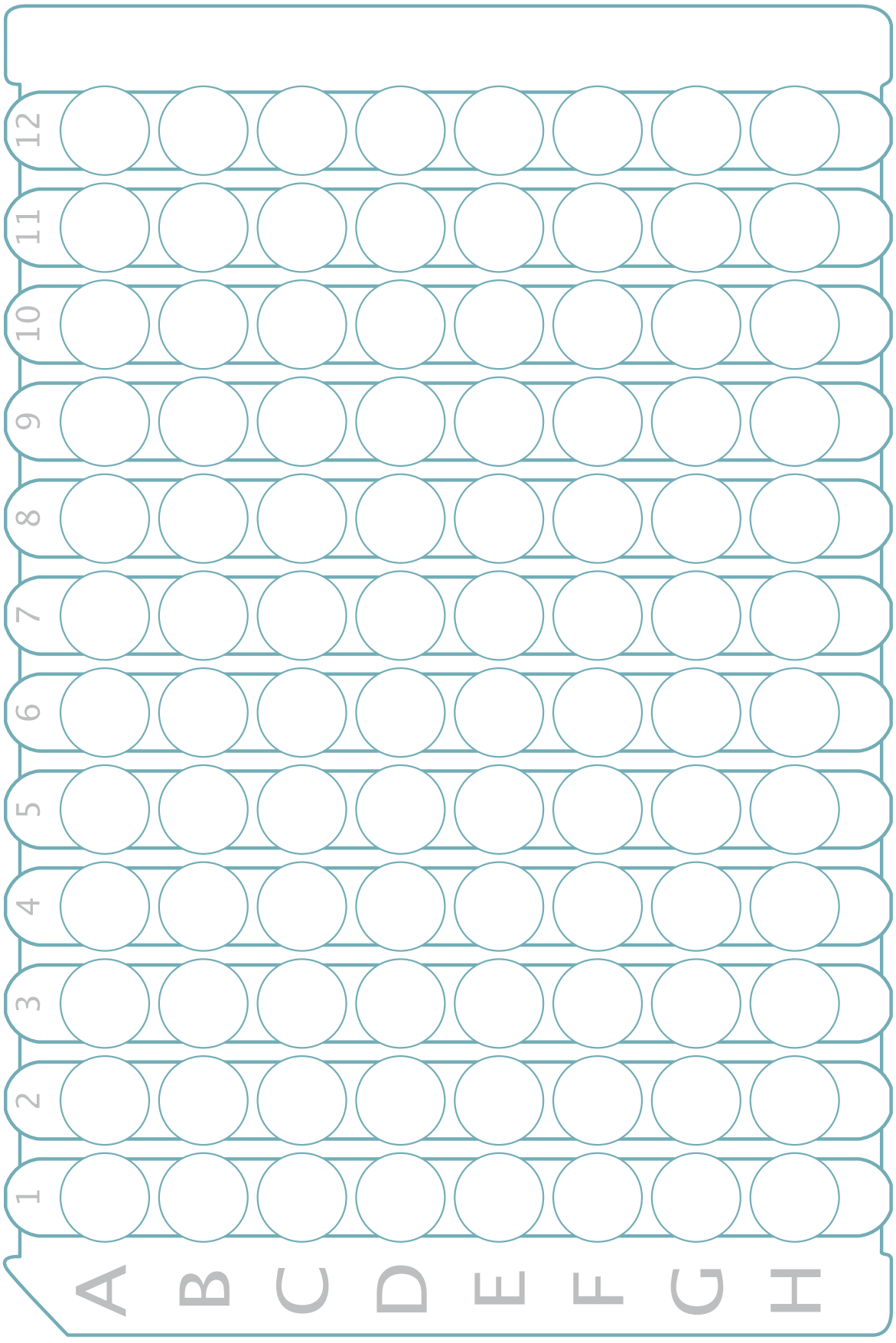
IGFBP-2
IGFBP-3
IGFBP-4

REFERENCES

1. Blundell, T.L. and R.E. Humbel (1980) Nature **287**:781.
2. Grimberg, A. and P. Cohen (2000) J. Cell. Physiol. **183**:1.
3. Bayes-Genis, A. *et al.* (2000) Circ. Res. **86**:125.
4. Hall, K. and V.R. Sara (1983) Vitamin. Horm. **40**:175.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES