Quantikine® ELISA

Human IGF-I/IGF-1 Immunoassay

Catalog Number DG100B
SG100B
PDG100B

For the quantitative determination of human Insulin-like Growth Factor 1 (IGF-1) concentrations in cell culture supernates, 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.

TABLE OF CONTENTS

SECTION PAGE

Manufactured and Distributed by:

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

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Insulin-like growth factor 1 (IGF-1, also known as somatomedin C) is a 7.6 kDa, 70 amino acid (aa) polypeptide with three internal disulfide bonds. The sequence of human IGF-1 is identical to that of bovine and porcine IGF-1, and is 70% identical to human IGF2. IGF-1 is a single-chain molecule with about 50% identity to the sequences of the A- and B-chains of human insulin (1-3).

IGF-1 is a growth hormone that plays a role in a variety of biological events. It 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. It binds two tyrosine kinase receptors, the IGF-1 receptor (IGF1R) and the insulin receptor, to initiate downstream events like the AKT and PI3K signal transduction pathways. This triggers cell proliferation and protects cells from apoptosis (4). IGF-1 also interacts with seven IGF-binding proteins (IGFBP-1 through IGFBP7), influencing IGF-1 binding to IGF1R and increasing IGF-1 half-life to closely regulate IGF-1 signaling (5). For example, 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. Proteases also facilitate IGF-1R binding by cleaving IGFBPs to modify their affinity for IGF or completely eliminate the IGFBP (6). 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 (7-10). Its ability to inhibit apoptosis and stimulate cell growth and proliferation plays a significant role in prenatal development, growth to adulthood, and metabolic control. Serum levels of IGF-1 have been reported to increase from birth to puberty, followed by a slow decline through adulthood (11). IGF-1 also induces amino acid uptake, protein synthesis, and glucose utilization. In the brain, IGF-1 acts as a neurotrophic factor to promote neurogenesis and neuronal survival (12, 13). Exercise increases levels of IGF-1 in blood serum, indicating it plays a role as a key mediator of exercise-induced neurogenesis (14).

The Quantikine® Human IGF-I/IGF-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IGF-1 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IGF-1 and has antibodies raised againt the recombinant protein. Results obtained for naturally occurring human IGF-1 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 natural human IGF-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IGF-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IGF-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IGF-1 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-1 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.
- If samples generate values higher than the highest standard, 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.
- 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.
- 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 # DG100B	CATALOG # SG100B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IGF-1 Microplate	899064	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IGF-1.	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.*
Human IGF-1 Standard	899066	2 vials	12 vials	Recombinant human IGF-1 in a buffer with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IGF-1 Conjugate	899065	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IGF-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-99	895031	1 vial	6 vials	11 mL/vial of a buffer with preservatives.	
Calibrator Diluent RD5-18	895335	2 vials	12 vials	21 mL/vial of a buffer with preservatives.	
Wash Buffer Concentrate	895003	2 vials	12 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.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DG100B contains sufficient materials to run an ELISA on one 96 well plate.
SG100B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDG100B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IGF-1 Microplate	899064	50 plates
Human IGF-1 Conjugate	899065	50 vials
Human IGF-1 Standard	899066	25 vials
Calibrator Diluent RD5-18	895335	100 vials
Assay Diluent RD1-99	895031	25 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate	895126	12 bottles
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	753136	2 booklets

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
- Human IGF-1 Controls (optional; R&D Systems®, Catalog # QC255)

PRECAUTIONS

IGF-1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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. Refer to the SDS 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: *Animal serum used in the preparation of cell culture media may contain high levels of animal IGF-1 that shares a high sequence homology. For best results, use serum-free media for growth of cell cultures during the last 24 hours when assaying for IGF-1 production.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 PREPARATION

Cell culture supernate samples require a 4-fold dilution due to matrix effect. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5-18.

Serum and plasma samples require a 100-fold dilution due to high endogenous levels. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-18.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

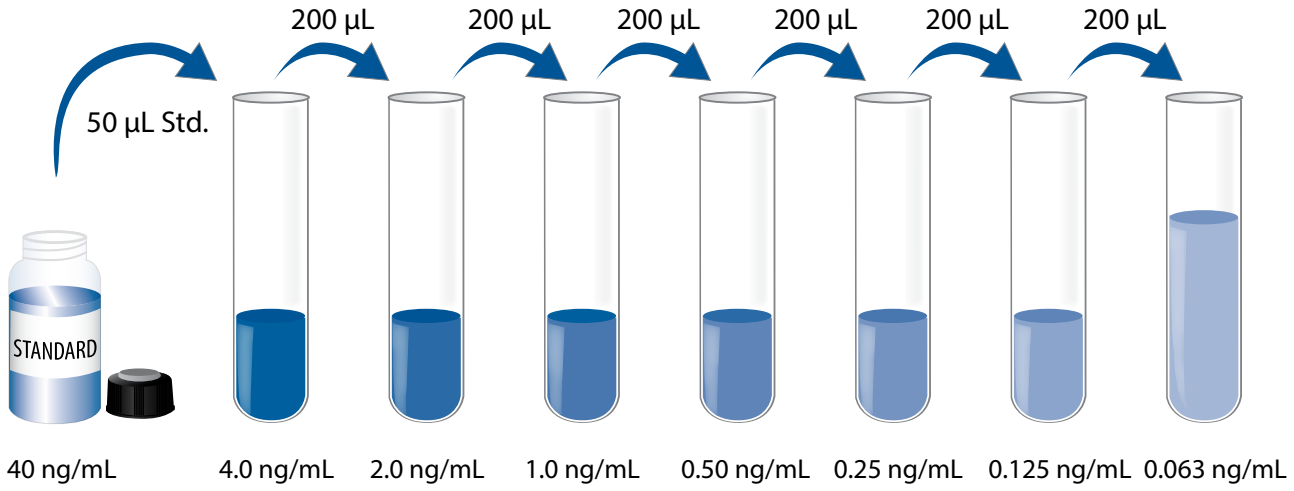
Note: *Concentrations of IGF-1 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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.

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.

Human IGF-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IGF-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-18 into the 4.0 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4.0 ng/mL standard serves as the high standard. Calibrator Diluent RD5-18 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, controls, and samples be assayed in duplicate.

Note: *Concentrations of IGF-1 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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. Wash and aspirate the plate a total of two times with Wash Buffer prior to assay. 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.
4. Add 50 µL of Assay Diluent RD1-99 to each well.
5. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. **Incubate for 3 hours at 2-8 °C. Do not stack plates.** A plate layout is provided to record standards and samples assayed.
6. 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.
7. Add 200 µL of Human IGF-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour at room temperature** on benchtop.
8. Repeat the aspiration/wash as in step 6.
9. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
10. 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.
11. 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 Sample Preparation section.

CALCULATION OF RESULTS

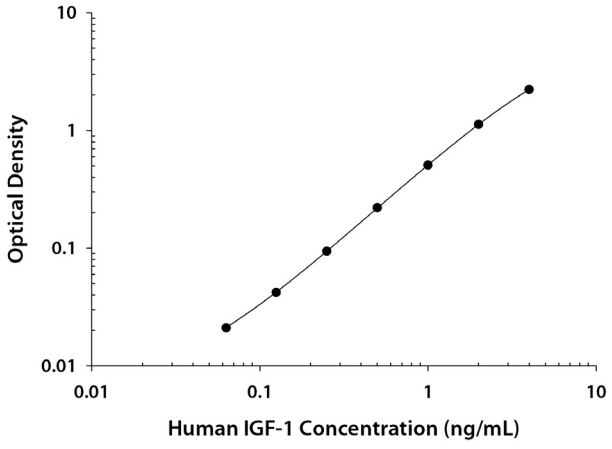
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human IGF-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Because 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	0.024 0.025	0.025	—
0.063	0.045 0.046	0.046	0.021
0.125	0.066 0.068	0.067	0.042
0.25	0.119 0.119	0.119	0.094
0.50	0.245 0.246	0.246	0.221
1.0	0.518 0.546	0.532	0.507
2.0	1.149 1.152	1.151	1.126
4.0	2.252 2.258	2.255	2.230

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)	0.594	1.02	1.94	0.617	1.01	2.10
Standard deviation	0.026	0.046	0.077	0.038	0.060	0.120
CV (%)	4.4	4.5	4.0	6.2	5.9	5.7

RECOVERY

The recovery of human IGF-1 spiked to three different levels throughout the range of the assay was evaluated. Samples were diluted prior to assay as described in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	93	88-97%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IGF-1 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as described in the Sample Preparation section.

		Serum-free cell culture media (n=4)	Serum (n=6)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	103	102	101	100
	Range (%)	99-110	100-104	97-104	98-105
1:4	Average % of Expected	100	102	97	96
	Range (%)	96-106	100-105	93-99	92-101
1:8	Average % of Expected	97	104	100	97
	Range (%)	93-105	101-110	94-104	93-102
1:16	Average % of Expected	99	110	103	101
	Range (%)	96-104	105-115	98-107	94-107

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human IGF-1 ranged from 0.004-0.022 ng/mL. The mean MDD was 0.010 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. 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-1 produced at R&D Systems®.

The NIBSC/WHO IGF-1 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-1 kit to approximate NIBSC/WHO 02/254 values, use the equation below.

NIBSC/WHO (02/254) approximate value (ng/mL) = 0.8685 x Quantikine® Human IGF-1 value (ng/mL)

Note: *Based on data generated in April 2019.*

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IGF-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=30)	117	58.8-201	37.2
EDTA plasma (n=30)	119	58.6-211	37.4
Heparin plasma (n=30)	118	62.4-232	39.0

Cell Culture Supernates:
HepG2 human hepatocellular carcinoma cells were grown in MEM NEAA Earl's Salts supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent (normoxia). For hypoxia, cells were incubated for an additional 72 hours in a hypoxia workstation set to 1% O_2. The cell conditioned media was taken off and centrifuged to remove any cells or debris. Aliquots of the cell culture supernates were removed and assayed for human IGF-1.

Condition	(ng/mL)
Normoxia	2.52
Hypoxia	6.59

SPECIFICITY

This assay recognizes natural and recombinant human IGF-1.

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 recombinant human IGF-1 standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Cyr61
IGF-1R
IGF-1R/Integrin α6β4 X1 Isoform Complex
IGF-1R/Integrin αVβ3 Complex
IGF2
IGF2R
IGFALS
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGFBP-7
Insulin R
Integrin α6β4 X1 Isoform
Integrin αVβ3
Proinsulin

Recombinant mouse:

IGF1
IGF2

Recombinant rat:

IGF-1

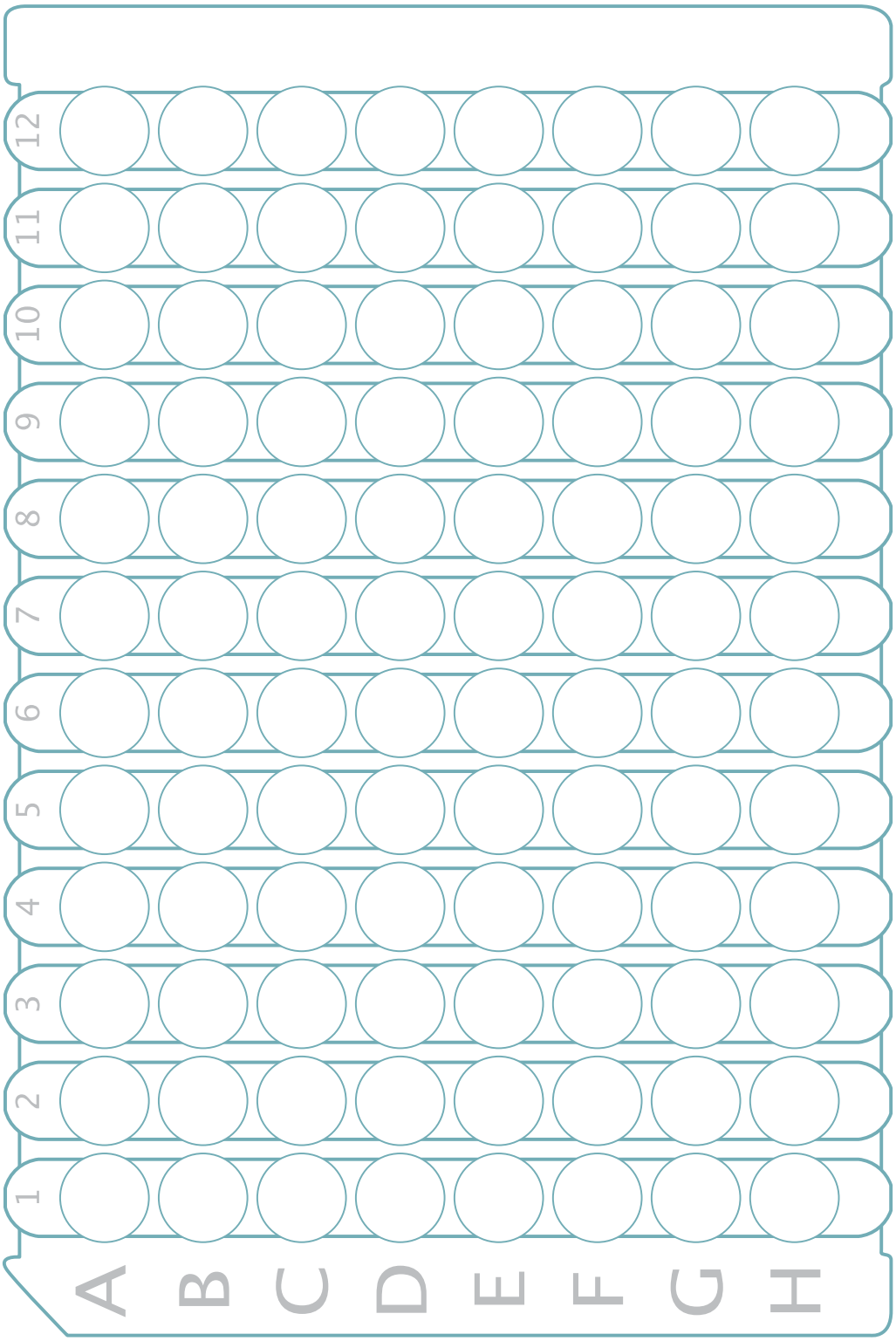
Recombinant human LR3 IGF-1 interferes at concentrations > 10 ng/mL and cross-reacts approximately 4.2% in this assay.

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. Peruzzi, F. *et al.* (1999) Mol Cell Biol. **19**:7203.
5. Allard, J.B. and C. Duan (2018) Front. Endrocrinol. **9**:117.
6. Mazerbourg, S. and P. Monget (2018) Front. Endrocrinol. **9**;134.
7. Hakuno, F. and S.I. Takahashi (2018) J. Mol. Endocrinol. **61**:T69.
8. Kasprzak, A. *et al.* (2017) Mutat. Res. Rev. Mutat. Res. **772**:78.
9. Sivasubramaniyam, T. (2017) JCI Insight. **2**:e93735.
10. Shigematsu, S. *et al.* (1999) Endocr. J. **46**:S59.
11. Hall, K. and V.R. Sara (1983) Vitamin. Horm. **40**:175.
12. Phillips, C. *et al.* (2014) Front. Cell Neurosci. **8:**170.
13. Aberg, D. (2010) Endocrine Development **17**:63.
14. Torres-Aleman, I. (2010) Dev. Neurobiol. **70**:384.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



All trademarks and registered trademarks are the property of their respective owners.