

# Quantikine<sup>®</sup> ELISA

## Human Free IGFBP-1 Immunoassay

Catalog Number DGB100

For the quantitative determination of human Free Insulin-like Growth Factor Binding Protein 1 (Free IGFBP-1) concentrations in cell culture supernates, cell lysates, serum, plasma, saliva, urine, and human milk.

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
INTRODUCTION .....	1
PRINCIPLE OF THE ASSAY.....	2
LIMITATIONS OF THE PROCEDURE .....	2
TECHNICAL HINTS.....	2
MATERIALS PROVIDED & STORAGE CONDITIONS .....	3
OTHER SUPPLIES REQUIRED .....	4
SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES .....	4
PRECAUTIONS.....	4
SAMPLE COLLECTION & STORAGE .....	5
SAMPLE PREPARATION.....	5
CELL LYSIS PROCEDURE.....	5
REAGENT PREPARATION.....	6
ASSAY PROCEDURE .....	7
CALCULATION OF RESULTS.....	8
TYPICAL DATA.....	8
PRECISION.....	9
RECOVERY.....	9
LINEARITY.....	10
SENSITIVITY .....	10
CALIBRATION .....	10
SAMPLE VALUES.....	11
SPECIFICITY.....	12
REFERENCES.....	13
PLATE LAYOUT.....	14

## 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

The superfamily of secreted insulin-like growth factor (IGF) binding proteins includes high-affinity IGF binding proteins (IGFBPs) and low-affinity binding proteins referred to as IGFBP-like (IGFBPL) or IGFBP-related proteins (IGFBP-rP) (1). IGFBPs form complexes with IGF-I and -II, modulating their activity and bioavailability by modifying their binding to cell receptors, cell surfaces, and the extracellular matrix (1-5). IGFBP-1 has specifically been shown to inhibit IGF action in bone (6). In addition to direct effects on activity and binding to IGFs, IGFBPs act as carriers for circulating IGFs, prolonging their half-lives, and creating IGF reservoirs in tissues (1, 3, 4). IGFBP-1 and other superfamily members contain N-terminal IGF-binding domains with 12 conserved cysteines and C-terminal thyroglobulin type 1 domains with 6 conserved cysteines (1).

IGFBP-1 is produced mainly in the liver and kidney, and is less abundantly expressed in other tissues, including the reproductive system (7, 8). IGFBP-1 levels are primarily regulated by changes in plasma insulin concentrations, possibly in part via C/EBP $\beta$  and Hedgehog signaling, which have also been shown to regulate IGFBP-1 expression (9-11). IGFBP-1 administration diminishes the hypoglycemic response to exogenous IGF-I (12-14). In addition to regulating IGFs, IGFBP-1 may also be involved in cell migration, as it has been shown to stimulate Focal Adhesion Kinase (FAK) through binding to integrins (15-17).

Both low and high expression levels of IGFBP-1 have been associated with several human pathologies. Low levels of IGFBP-1 are linked to insulin resistance, and low fasting levels of IGFBP-1 are associated with diabetes development in Swedish men (18-24). Conversely, increased IGFBP-1 levels are associated with coronary heart disease as well as long term morbidity and mortality following acute myocardial infarction (AMI) (25, 26). Elevated IGFBP-1 levels are also associated with more rapid progression to castration resistant prostate cancer and lower overall survival in men with metastatic prostate cancer (27). IGFBP-1 may also aid in the early detection of alcohol-induced liver injury and the confirmation of premature rupture of the membranes (PROM) in pregnant women (28, 29).

The Quantikine Human Free IGFBP-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human free IGFBP-1 in cell culture supernates, cell lysates, serum, plasma, urine, and human milk. It contains NS0-expressed recombinant human IGFBP-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IGFBP-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 naturally occurring human free IGFBP-1.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Free IGFBP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IGFBP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Free IGFBP-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 Free IGFBP-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, further dilute the samples with Calibrator Diluent and repeat the assay.
- Samples, controls, and standards must be pipetted within 15 minutes.
- 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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
<b>Human IGFBP-1 Microplate</b>	894812	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Free IGFBP-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.*
<b>Human IGFBP-1 Standard</b>	894814	2 vials of human IGFBP-1 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Discard after use. Use a new standard for each assay.
<b>Human IGFBP-1 Conjugate</b>	894813	21 mL of a polyclonal antibody specific for human Free IGFBP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
<b>Assay Diluent RD1-14</b>	895180	11 mL of a buffered protein base with and preservatives. <i>May contain a precipitate. Mix well before and during use.</i>	
<b>Calibrator Diluent RD5P Concentrate</b>	895151	21 mL of a concentrated buffered protein base with preservatives. <i>Use diluted 1:5 in this assay.</i>	
<b>Wash Buffer Concentrate</b>	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
<b>Color Reagent A</b>	895000	12 mL of stabilized hydrogen peroxide.	
<b>Color Reagent B</b>	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
<b>Stop Solution</b>	895032	6 mL of 2 N sulfuric acid.	
<b>Plate Sealers</b>	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.
- Human Free IGFBP-1 Controls (optional; R&D Systems, Catalog # QC204).

## SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems, Catalog # 890713)
- PBS

## PRECAUTIONS

IGFBP-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 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.

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

## 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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Cell Lysates** - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

**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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay  
Grossly icteric samples are not suitable for use in this assay.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Human Milk** - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Serum and plasma samples may require up to a 10-fold dilution due to the broad sample value range. A suggested 10-fold dilution is 30  $\mu$ L of sample + 270  $\mu$ L of Calibrator Diluent RD5P (diluted 1:5)\*.

Human milk samples require a 10-fold dilution. A suggested 10-fold dilution is 30  $\mu$ L of sample + 270  $\mu$ L of Calibrator Diluent RD5P (diluted 1:5).

## CELL LYSIS PROCEDURE

**Use the following procedure for the preparation of cell lysate samples.**

1. Wash cells three times in cold PBS.
2. Resuspend cells a  $1 \times 10^7$  cells/mL in Cell Lysis Buffer 1.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at 8000 x g for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at  $\leq -70$  °C until ready for use.

\*See Reagent Preparation section.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

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

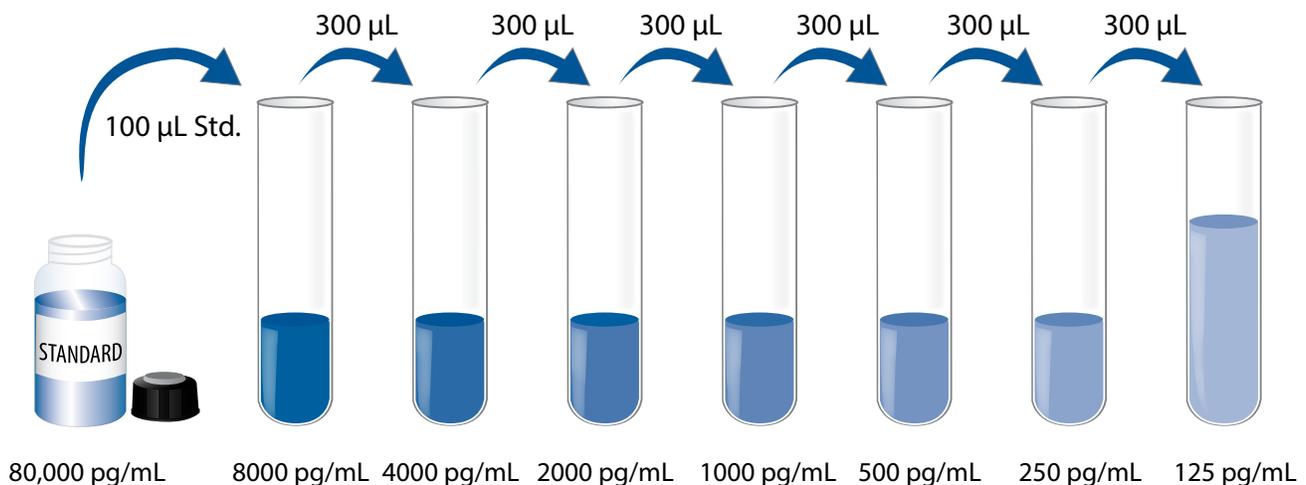
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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

**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).

**Human IGFBP-1 Standard - Refer to the vial label for reconstitution volume.** Reconstitute the Human IGFBP-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 80,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900  $\mu$ L of Calibrator Diluent RD5P (diluted 1:5) into the 8000 pg/mL tube. Pipette 300  $\mu$ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, controls, and standards be assayed in duplicate.**

**Note:** *IGFBP-1 is 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. Add 50  $\mu$ L of Assay Diluent RD1-14 to each well. *Assay Diluent RD1-14 may contain a precipitate. Mix well before and during use.*
4. Add 50  $\mu$ L of Standard, control, or sample\* per well. Cover with the adhesive strip provided. Incubate for 3 hours **at 2-8 °C**. A plate layout is provided to record standards and samples assayed.

**Note:** *Standard, control, and sample 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  $\mu$ L) 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  $\mu$ L of Human IGFBP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour **at room temperature**.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu$ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or 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.

\*Samples may require dilution. See the Sample Preparation section.

## CALCULATION OF RESULTS

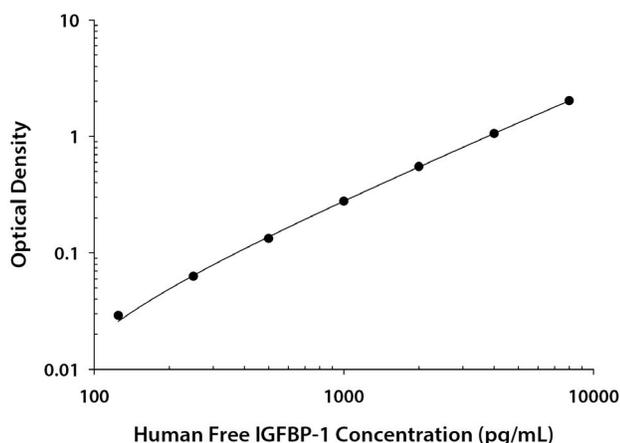
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human IGFBP-1 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.



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.011	0.011	—
125	0.039 0.041	0.040	0.029
250	0.071 0.076	0.074	0.063
500	0.143 0.145	0.144	0.133
1000	0.280 0.297	0.289	0.278
2000	0.558 0.566	0.562	0.551
4000	1.062 1.073	1.068	1.057
8000	2.017 2.062	2.040	2.029

## 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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	1038	3065	5639	1079	3100	5895
Standard deviation	58.2	131	230	103	209	373
CV (%)	5.6	4.3	4.1	9.5	6.7	6.3

## RECOVERY

The recovery of human Free IGF1P-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	112	92-117%
Cell lysate (n=4)	107	91-117%
Serum (n=4)	94	86-107%
EDTA plasma (n=4)	97	86-111%
Heparin plasma (n=4)	97	85-108%
Human milk (n=4)	104	92-114%

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Free IGFBP-1 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Cell lysate (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	104	105	97	103	102	104	107
	Range (%)	102-106	97-111	94-100	97-107	98-107	100-109	99-114
1:4	Average % of Expected	102	101	97	107	104	105	105
	Range (%)	96-107	89-107	93-103	100-111	97-108	99-108	100-109
1:8	Average % of Expected	101	99	97	107	110	107	104
	Range (%)	93-111	83-105	92-102	99-111	106-116	98-115	94-114
1:16	Average % of Expected	101	96	96	106	111	107	101
	Range (%)	89-115	89-101	91-103	97-111	105-124	99-116	85-115

\*Samples were diluted prior to assay.

## SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of human Free IGFBP-1 ranged from 3.73-13.8 pg/mL. The mean MDD was 6.36 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.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human IGFBP-1 produced at R&D Systems.

## SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	12,952	499-62,671	14,097
EDTA plasma (n=36)	11,186	372-58,113	12,693
Heparin plasma (n=36)	8126	199-50,156	10,479
Urine (n=12)	524	139-1257	380
Human milk (n=10)	15,045	2535-28,056	8236

### Cell Culture Supernates/Cell Lysates:

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and grown until confluent.

U-87 MG human glioblastoma/astrocytoma cells were cultured in MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and grown until confluent.

HUVEC human umbilical vein endothelial cells were cultured in EGM-2 and grown until confluent.

Aliquots of the cell culture supernates were removed and assayed for human free IGFBP-1. Cells were lysed and assayed for human Free IGFBP-1.

Cell Line	Cell Culture Supernate (pg/mL)	Cell Lysate (pg/mg)
HepG2	533,171	242,820
U-87 MG	21,283	12,302
HUVEC	150	ND

ND=Non-detectable

## SPECIFICITY

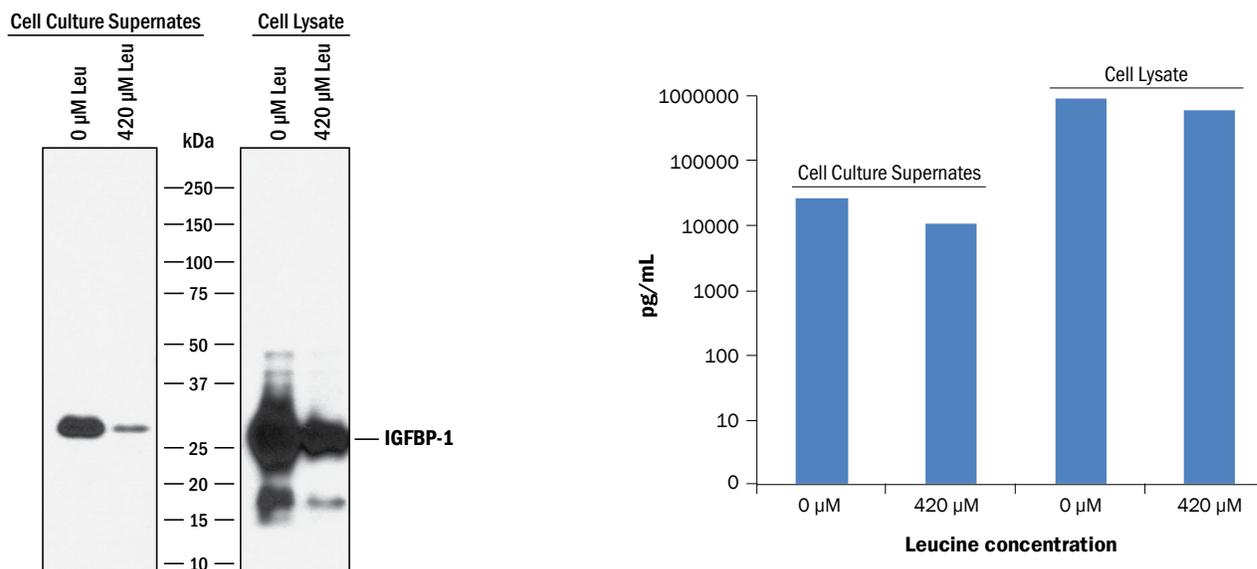
This assay recognizes natural and recombinant phosphorylated and non-phosphorylated human free IGFBP-1. This assay does not detect IGFBP-1 in complex with IGF-I or IGF-II.

The factors listed below were prepared at 80 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 80 ng/mL in a mid-range human free IGFBP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

IGFBP-2  
IGFBP-3  
IGFBP-4  
IGFBP-5  
IGFBP-6  
IGFBP-7  
IGFBP-L1  
IGFBP-rP10

Recombinant mouse IGFBP-1 cross-reacts approximately 0.15% in this assay.



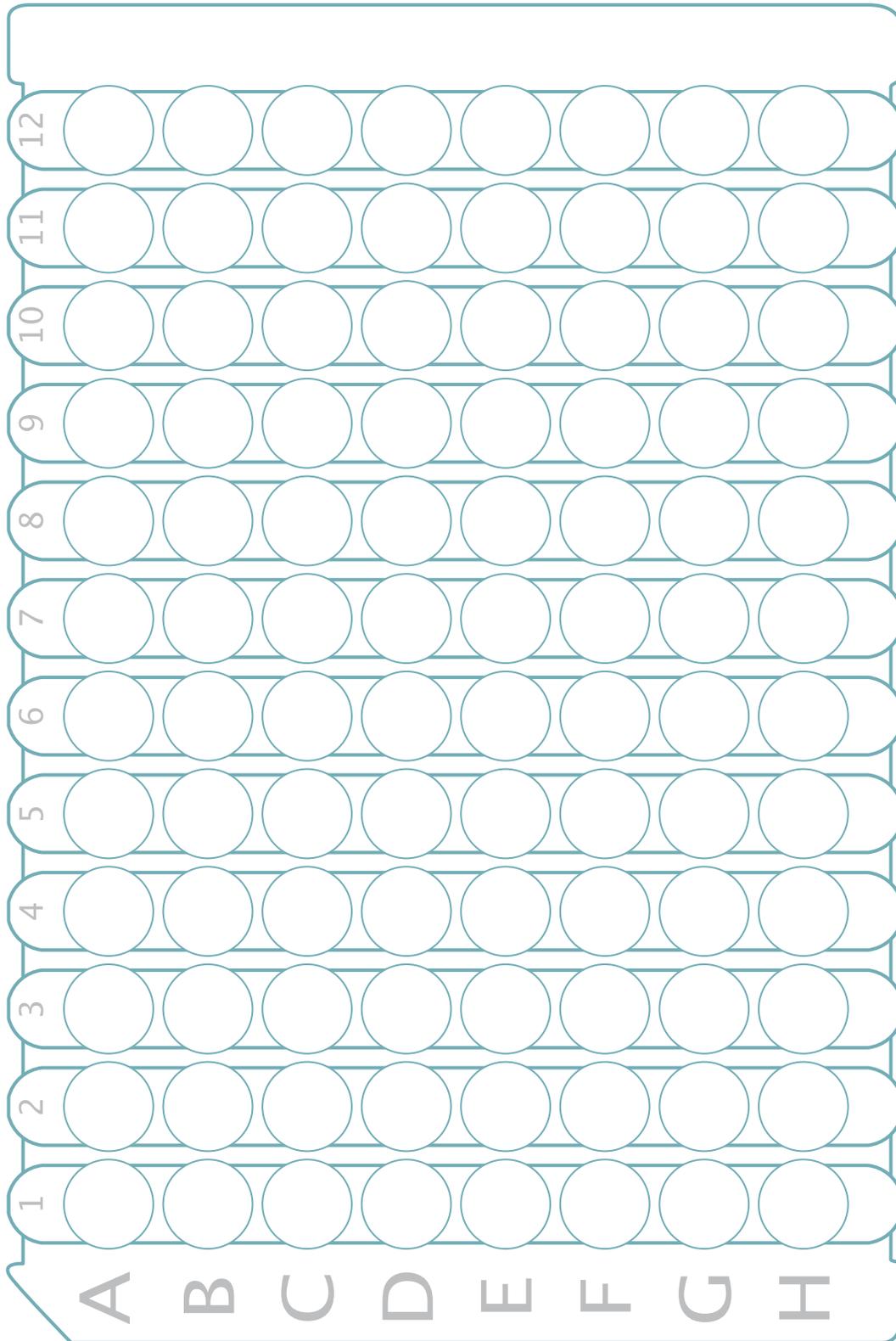
HepG2 conditioned media and lysate samples were analyzed by Western blot and this Quantikine ELISA. The HepG2 cells were serum-starved and then incubated in the absence or presence of the amino acid leucine. Equal volumes of cell culture supernates and amounts of total cell protein (Lysate) samples, respectively, 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 relative correlation with the ELISA sample value.

## REFERENCES

1. Duan, C. and Q. Xu (2005) *Gen. Comp. Endocrinol.* **142**:44.
2. Lee, W.H. *et al.* (1992) *J. Neurosci.* **12**:4737.
3. Chesik, D. *et al.* (2007) *Cytokine Growth Factor Rev.* **18**:267.
4. Rajaram, S. *et al.* (1997) *Endocr. Rev.* **18**:801.
5. Arai, T. *et al.* (1996) *Endocrinology* **137**:4571.
6. Govoni, K.E. (2012) *Curr. Mol. Pharmacol.* **5**:143.
7. Lee, P.D. *et al.* (1993) *Proc. Soc. Exp. Biol. Med.* **204**:4.
8. Lee, P.D. *et al.* (1997) *Proc. Soc. Exp. Biol. Med.* **216**:319.
9. Yki-Järvinen, H. *et al.* (1995) *J. Clin. Endocrinol. Metab.* **80**:3227.
10. Tamura, I. *et al.* (2014) *Endocrinology* **155**:275.
11. Matz-Soja, M. *et al.* (2014) *Cell Commun. Signal.* **12**:11.
12. Lewitt, M.S. *et al.* (1991) *Endocrinology* **129**:2254.
13. Crossey, P.A. *et al.* (2000) *Diabetes* **49**:457.
14. Mortensen, D.L. *et al.* (1997) *Endocrinology* **138**:2073.
15. Frost, R.A. and C.H. Lang (1999) *Endocrinology* **140**:3962.
16. Perks, C.M. *et al.* (1999) *J. Mol. Endocrinol.* **22**:141.
17. Ammoun, S. *et al.* (2012) *Oncogene* **31**:1710.
18. Conway, G.S. *et al.* (1990) *Clin. Endocrinol. (Oxf)* **33**:593.
19. Weaver, J.U. *et al.* (1990) *Clin. Endocrinol. (Oxf)* **33**:415.
20. Buyalos, R.P. *et al.* (1995) *Am. J. Obstet. Gynecol.* **172**:932.
21. Mogul, H.R. *et al.* (1996) *J. Clin. Endocrinol. Metab.* **81**:4492.
22. Kaushal, K. *et al.* (2004) *Diabetes Care* **27**:2682.
23. Lewitt, M.S. *et al.* (2008) *Diabetologia* **51**:1135.
24. Gu, T. *et al.* (2013) *Clin. Epigenetics* **5**:21.
25. Prentice, R.L. *et al.* (2013) *Genome Med.* **5**:112.
26. Hui, L. *et al.* (2011) *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* **33**:22.
27. Sharma, J. *et al.* (2014) *Prostate* **74**:225.
28. Li, H.H. *et al.* (2013) *J. Transl. Med.* **11**:266.
29. Abdelazim, I.A. (2014) *J. Obstet. Gynaecol. Res.* **40**:961.

## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



©2014 R&D Systems, Inc.

753022.0