Quantikine® ELISA

Human IGFBP-2 Immunoassay

Catalog Number DGB200

For the quantitative determination of Insulin-like Growth Factor Binding Protein 2 (IGFBP-2) concentrations in cell culture supernates, serum, plasma, and urine.

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	3
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
CALIBRATION	8
LINEARITY	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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 (IGFBP) and low-affinity binding proteins referred to as IGFBP-like or IGFBP-related proteins (IGFBPL, 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). In addition to direct effects on activity and binding of IGFs, IGFBPs act as carriers for circulating IGFs, prolonging their half-lives, and creating IGF reservoirs in tissues (1, 3, 4). IGFBP-2 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). The C-terminal domain mediates heparin binding, which is enhanced in the presence of IGFs and, in the case of IGFBP-2, contains an RGD sequence that promotes integrin-mediated cell attachment (1, 3, 5-9). IGFBP-2 affinity and extracellular matrix binding enhancement is stronger for IGF-II than IGF-I (4, 9).

IGFBP-2 is expressed in a number of tissues during development, with highest expression levels in the central nervous system (CNS) (2-4, 10). Overall IGFBP-2 expression is lower in adults, and occurs mainly in the CNS and reproductive tissues (2-4). Specific CNS expression is reported in Bergmann glia in the cerebellum, and Müller cells and astrocytes in the retina (2-5). IGFBP-2 is the major family member in cerebrospinal fluid, and can be upregulated in pathological conditions including multiple sclerosis (3, 4). After IGFBP-3, IGFBP-2 is the next most abundant IGFBP in serum or plasma (3, 4, 10). IGFBP-2 is also found in body fluids including seminal plasma, amniotic, follicular, lymphatic and synovial fluids, milk, and urine (3, 4).

IGFBP-2 modulates the effects of IGF-I on myelinogenesis and neuronal differentiation and survival (3). It modulates the role of IGF- II as a uterine epithelial mitogen, and high concentrations of IGFBP-2 in umbilical cord serum have been correlated with fetal intrauterine growth restriction (11, 12). IGFBP-2 expression by bone marrow stromal cells is important for their support of hematopoietic stem cells (13). Circulating IGFBP-2 concentrations can be modulated by IGFs, glucocorticoids (which can suppress IGFBP-2 expression), and insulin (which enhances its production by white adipocytes) (3, 4, 14-17). Increased serum concentrations of IGFBP-2 are reported in uncontrolled diabetes, chronic renal failure, excesses of growth hormone (acromegaly), and undernutrition (4). In prostate cancer, acute myeloid leukemia, rhabdomyosarcoma, Wilm's tumor in the kidney, CNS tumors such as glioblastoma, and other cancers, serum or spinal fluid concentration often correlates with malignancy or chemotherapy resistance (3, 4, 7, 10, 17-19).

IGFBP-2 is thought to have a protective role against obesity and insulin resistance, most likely by inhibiting adipocyte differentiation and limiting IGF bioavailability (9, 14-16, 20). Plasma concentration correlates inversely with body mass index, plasma insulin, and markers of insulin resistance (14, 20). Deletion of IGFBP-2 in male mice shows reduced bone mass, probably due to IGFBP-2 support of osteoblast differentiation, survival, proliferation, and balance with osteoclasts (21, 22). Binding of IGFBP-2 to integrin $\alpha 5\beta 1$ mediates IGF-independent enhancement of cell mobility, while binding to $\alpha v\beta 3$ on breast tumor cells enhances vitronectin adhesion and reduces IGF-mediated growth and migration (6-8). Cleavage of IGFBP-2 by cancer cell-secreted metalloproteinases such as MMP-7 can release IGFs, which then promote cancer cell growth (23).

The Quantikine Human IGFBP-2 Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human IGFBP-2 in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human IGFBP-2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IGFBP-2 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 IGFBP-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IGFBP-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IGFBP-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IGFBP-2 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 IGFBP-2 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.
- 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
Human IGFBP-2 Microplate	892138	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IGFBP-2.	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 IGFBP-2 Conjugate	892139	21 mL of polyclonal antibody specific for human IGFBP-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IGFBP-2 Standard	892140	Recombinant human IGFBP-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature and mix well to dissolve.*	
Calibrator Diluent RD5-20	895346	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human IGFBP-2 Controls (R&D Systems, Catalog # QC106).

PRECAUTIONS

IGFBP-2 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. 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.

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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 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.*

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

SAMPLE PREPARATION

Serum and plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 10 μL of sample + 490 μL of Calibrator Diluent RD5-20.

Urine samples require a 5-fold dilution. A suggested 5-fold dilution is 40 μL of sample + 160 μL of Calibrator Diluent RD5-20.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

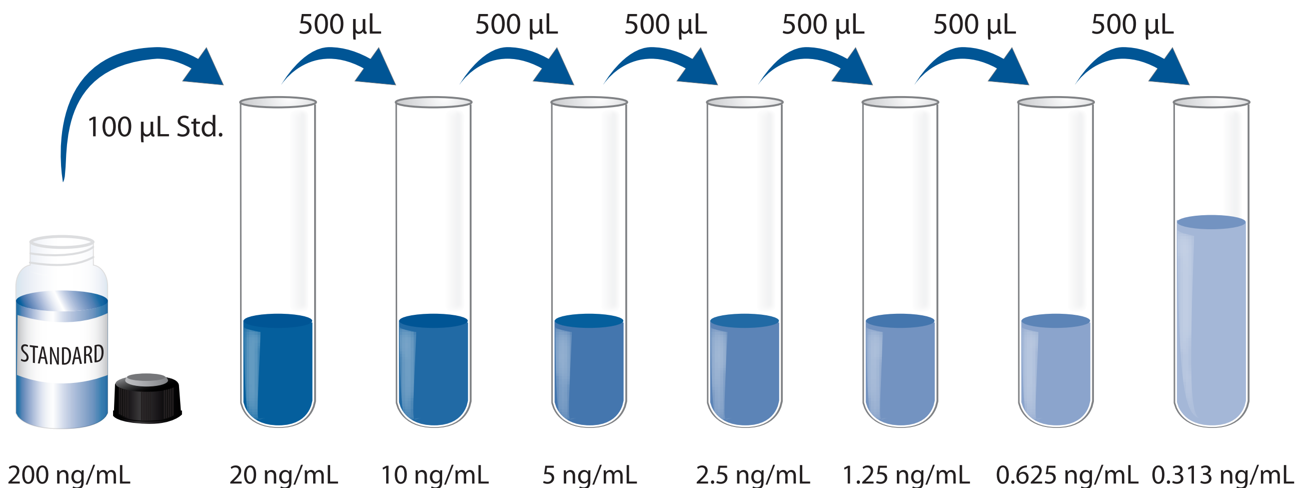
Note: *IGFBP-2 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 µL of the resultant mixture is required per well.

Human IGFBP-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IGFBP-2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-20 into the 20 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. Calibrator Diluent RD5-20 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, samples, and controls be assayed in duplicate.

Note: *IGFBP-2 is detectable 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 100 µL of Assay Diluent RD1X to each well. *Assay Diluent RD1X may contain crystals. Warm to room temperature and mix well to dissolve before use.*
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process 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 Human IGFBP-2 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 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 µL 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 Sample Preparation section.

CALCULATION OF RESULTS

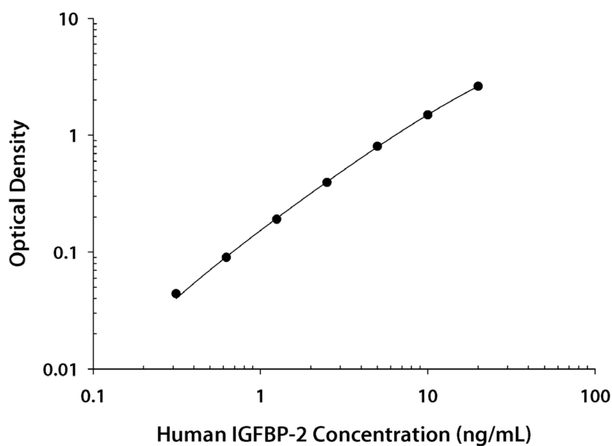
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-2 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.



(ng/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
0.313	0.057 0.058	0.058	0.044
0.625	0.100 0.107	0.104	0.090
1.25	0.199 0.211	0.205	0.191
2.5	0.399 0.419	0.409	0.395
5	0.809 0.824	0.817	0.803
10	1.507 1.507	1.507	1.493
20	2.555 2.712	2.634	2.620

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)	1.96	6.24	12.7	2.24	6.93	13.6
Standard deviation	0.07	0.31	0.63	0.17	0.31	0.62
CV (%)	3.6	5.0	5.0	7.6	4.5	4.6

RECOVERY

The recovery of human IGFBP-2 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	100	88-113%
Urine* (n=4)	109	94-117%

*Samples were diluted prior to assay.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human IGFBP-2 ranged from 0.01-0.09 ng/mL. The mean MDD was 0.04 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 NS0-expressed recombinant human IGFBP-2 produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human IGFBP-2 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	93	105	101	103	100
	Range (%)	90-96	98-112	98-105	92-114	98-102
1:4	Average % of Expected	93	107	104	107	105
	Range (%)	88-102	96-118	101-106	102-114	101-110
1:8	Average % of Expected	102	103	105	112	106
	Range (%)	96-108	97-110	100-109	107-117	102-113
1:16	Average % of Expected	107	107	106	111	103
	Range (%)	98-112	102-114	99-111	102-119	94-111

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human IGFBP-2 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=35)	131	37-262	62
EDTA plasma (n=35)	126	33-276	58
Heparin plasma (n=35)	140	34-302	65
Urine (n=12)	25	6.6-41	11

Cell Culture Supernates:

MDA-MB-453 human breast cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum, and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for levels of natural human IGFBP-2, and measured 51 ng/mL.

IMR-32 human neuroblastoma cells were cultured in MEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for levels of natural human IGFBP-2, and measured 15 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IGFBP-2.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human IGFBP-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IGF-I R
IGF-II R
IGFBP-1
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGFBP-7
Integrin α3β1/VLA-3
Integrin α5β1
Integrin αVβ3

Recombinant mouse:

IGFBP-2

The following recombinant factors did not cross-react with IGFBP-2 in this immunoassay, but demonstrated a low level of interference. These factors were added at various concentrations to a mid level IGFBP-2 control. The chart below lists the percentage the IGFBP-2 control decreased out of the +/-3 SD range in the presence of the indicated concentration of interfering factor:

Concentration	Human IGF-I	Human IGF-II
50 ng/mL	6.9%	4.1%
100 ng/mL	8.1%	4.4%
200 ng/mL	7.5%	7.0%

For research use only. Not for use in diagnostic procedures.

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) Endocrine Rev. **18**:801.
5. Arai, T. *et al.* (1996) Endocrinology **137**:4571.
6. Wang, G.K. *et al.* (2006) J. Biol. Chem. **281**:14085.
7. Frommer, K.W. *et al.* (2006) J. Mol. Endocrinol. **37**:13.
8. Periera, J.J. *et al.* (2004) Cancer Res. **64**:977.
9. Conover, C.A. *et al.* (2003) Growth Horm. IGF Res. **13**:328.
10. Fukushima, T. and H. Kataoka (2007) Anticancer Res. **27**:3685.
11. Badinga, L. *et al.* (1999) J. Mol. Endocrinol. **23**:277.
12. Smerieri, A. *et al.* (2011) PLoS One **6**:e29562.
13. Huynh, H.D. *et al.* (2011) Blood **118**:3236.
14. Wheatcroft, S.B. and M.T. Kearney (2009) Trends Endocrinol. Metab. **20**:153.
15. Wheatcroft, S.B. *et al.* (2007) Diabetes **56**:285.
16. Li, Z. *et al.* (2012) Mol. Cell. Endocrinol. **358**:63.
17. Hoeflich, A. *et al.* (2001) Cancer Res. **61**:8601.
18. Kuhnl, A. *et al.* (2011) Leuk. Res. **35**:1585.
19. Tombolan, L. *et al.* (2011) Am. J. Pathol. **179**:2611.
20. Ko, J.M. *et al.* (2012) Endocr. J. **59**:335.
21. Kawai, M. *et al.* (2011) J. Biol. Chem. **286**:14670.
22. DeMambro, V.E. *et al.* (2008) J. Bone Miner. Res. **27**:390.
23. Miyamoto, S. *et al.* (2007) Cancer Sci. **98**:685.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

For research use only. Not for use in diagnostic procedures.

NOTES

NOTES

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

©2016 R&D Systems, Inc.