Quantikine® ELISA

Mouse IGFBP-3 Immunoassay

Catalog Number MGB300

For the quantitative determination of mouse Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) concentrations in cell culture supernates and plasma.

Note: 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

Insulin-like growth factor binding protein 3 (IGFBP-3) is a 40-44 kDa secreted glycoprotein that belongs to the IGFBP family of proteins (1-3). Mouse IGFBP-3 is synthesized as a 291 amino acid (aa) precursor that contains a 27 aa signal sequence and a 264 aa mature chain (Swiss Prot. #: P47878). Like all the IGFBPs, IGFBP-3 contains conserved amino- and carboxy-terminal domains that contain 18 cysteine residues (1). The central domain of the glycoprotein shows more variability and may be involved in actions unique to IGFBP-3 (1, 4). The mature protein also contains three potential sites of N-linked glycosylation. Mouse IGFBP-3 shares 93% and 81% aa sequence identity with rat and human IGFBP-3, respectively (1). *In situ* hybridization shows that mRNA of mouse IGFBP-3 is detectable in embryos at 11.5 days gestation (1). The levels of mRNA expression slowly increase to a maximum at one week postnatally and are mainly detected in the kidney, liver, lung, heart, spleen, and skeletal muscle (1). In humans, serum concentrations of IGFBP-3 vary with age; low at birth, increasing during childhood, peaking during puberty, and decreasing thereafter (5-6).

IGFBP-3 is the most abundant of the IGFBPs in postnatal serum, and acting via IGF-dependent and IGF-independent mechanisms, it has been proven to be a growth inhibitory, apoptosis-inducing molecule (6). It binds IGF-I and IGF-II in association with acid labile subunit (ALS), and this 150 kDa ternary complex carries almost all the IGFs found in serum (6-7). IGFBP-3 can also inhibit IGF-I and IGF-II action on cells by binding them and sequestering them, thus preventing their interaction with the type I IGF receptor (6, 8-10). In this way, IGFBP-3 has an anti-proliferative effect (11). The proteolytic fragment of IGFBP-3 binds to IGFs with lower affinity.

In addition to its role of modulating IGF function, IGFBP-3 has direct IGF-independent effects on cellular functions. The IGF-independent effects are mediated through binding to matrix, cell-surface, cytoplasmic, nuclear, and mitochondrial molecules (12). IGFBP-3 modulates signaling via nuclear receptors such as retinoid X receptor and regulates both the intrinsic and extrinsic apoptosis pathways (12). Epidemiological studies have shown IGFBP-3 to be protective against many malignancies including prostate cancer (13-14), colorectal cancer (15-17), lung cancer (18), bladder cancer (19), and childhood leukemia (20). Studies on IGFBP-3 and breast cancer risk are controversial, some of which state an increased risk with increased levels of IGFBP-3 (21-22).

The actual mechanism for these IGF-independent effects remains poorly understood. IGFBP-3 binds to a series of diverse molecules. These include matrix molecules such as the TGF-β binding protein 1 and type I collagen, and cell-surface receptors such as LRP1 and transferrin which subsequently binds to the transferrin receptor for rapid internalization into the cell (23-25). IGFBP-3 can sometimes induce apoptosis via non-nuclear pathways (26), however, its interactions with retinoid X receptor seems to be necessary to its actions in some models (27). IGFBP-3 has also been described to interact with mitochondrial apoptosis regulators such as Nur77 (28) and to inhibit the Bax-antagonist humanin (29). Also, phosphorylation of intracellular IGFBP-3 may be essential for its actions (30).

The Quantikine Mouse IGFBP-3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IGFBP-3 in cell culture supernates and plasma. It contains NS0-expressed recombinant mouse IGFBP-3 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IGFBP-3 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring mouse IGFBP-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IGFBP-3 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse IGFBP-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IGFBP-3 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse IGFBP-3 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in 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.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IGFBP-3 Microplate	893034	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IGFBP-3.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IGFBP-3 Standard	893036	2 vials of recombinant mouse IGFBP-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh Standard and Control for each assay. Discard after use.
Mouse IGFBP-3 Control	893037	2 vials of recombinant mouse IGFBP-3 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse IGFBP-3 Conjugate	893035	12 mL of a polyclonal antibody specific for mouse IGFBP-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-40	895513	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
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	895174	23 mL of diluted hydrochloric 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.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge samples for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Serum and heparin plasma samples are not recommended for use in this assay. Sample values vary depending upon the time and/or temperature of collection and storage. Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Plasma samples require a 300-fold dilution. A suggested 300-fold dilution can be achieved by adding 10 µL of sample to 90 µL of Calibrator Diluent RD5-26 (diluted 1:4)*. Complete the 300-fold dilution by adding 10 µL of the diluted sample to 290 µL of Calibrator Diluent RD5-26 (diluted 1:4).

*See Reagent Preparation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IGFBP-3 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

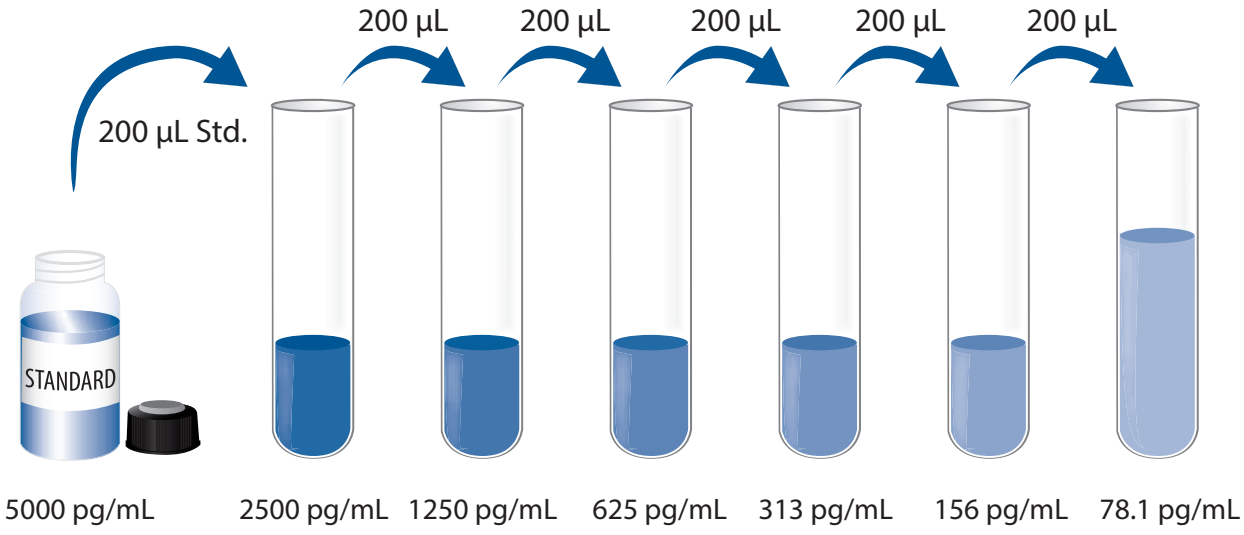
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse IGFBP-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IGFBP-3 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 5000 pg/mL. Allow the stock solution to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse IGFBP-3 Standard (5000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) 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 standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 50 µL of Assay Diluent RD1-40 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse IGFBP-3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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

CALCULATION OF RESULTS

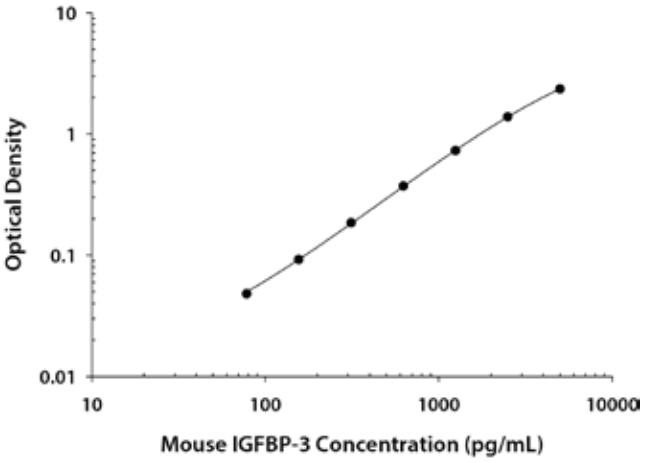
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse IGFBP-3 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	0.031 0.032	0.032	—
78.1	0.078 0.081	0.080	0.048
156	0.123 0.125	0.124	0.092
313	0.211 0.222	0.217	0.185
625	0.395 0.412	0.404	0.372
1250	0.750 0.772	0.761	0.729
2500	1.409 1.426	1.418	1.386
5000	2.377 2.390	2.384	2.352

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. 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	40	40	40
Mean (pg/mL)	280	628	2653	283	602	2648
Standard deviation	13.5	25.1	148	23.4	44.4	156
CV (%)	4.8	4.0	5.6	8.3	7.4	5.9

RECOVERY

The recovery of mouse IGFBP-3 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	100	90-109%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse IGFBP-3 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	EDTA plasma (n=5)
1:2	Average % of Expected	100	97
	Range (%)	98-102	86-105
1:4	Average % of Expected	100	101
	Range (%)	95-104	95-107
1:8	Average % of Expected	104	102
	Range (%)	93-118	95-106
1:16	Average % of Expected	102	99
	Range (%)	91-113	93-104

SENSITIVITY

Forty-four assays were evaluated and the minimum detectable dose (MDD) of mouse IGFBP-3 ranged from 2.74-16.0 pg/mL. The mean MDD was 7.95 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 mouse IGFBP-3 produced at R&D Systems.

SAMPLE VALUES

Plasma - Samples were evaluated for the presence of mouse IGFBP-3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
EDTA plasma (n=20)	364	211-498	71

Cell Culture Supernates - Mouse tissues were homogenized and seeded in RPMI 1640 supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate. Aliquots of the cell culture supernates were removed and assayed for levels of mouse IGFBP-3.

Tissue Type	# Mice Used	Seeded Volume	Incubation Time	Detectable Levels (pg/mL)	Stimulants
Lung	3	100 mL	3 days	16,380	10 µg/mL ConA
Kidney	8	55 mL	24 hours	15,965	None
Liver	1	100 mL	3 days	2022	None
Heart	4	85 mL	24 hours	1686	None
Pancreas	2	40 mL	4 days	1053	None
Spleen	4	100 mL	18 hours	770	None
Brain	4	100 mL	18 hours	182	None

SPECIFICITY

This assay recognizes natural and recombinant mouse IGFBP-3.

The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range mouse IGFBP-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

ALS
IGF-I
IGFBP-1
IGFBP-2
IGFBP-5
IGFBP-6
IGFBP-7
IGFBP-L1
IGFBP-L3
IGFBP-RP10

Recombinant human:

IGF-II R
IGFBP-3

This kit detects full-length and fragmented mouse IGFBP-3. It also detects IGF-I, IGF-II, IGF-I/ALS, and IGF-II/ALS complexed IGFBP-3.

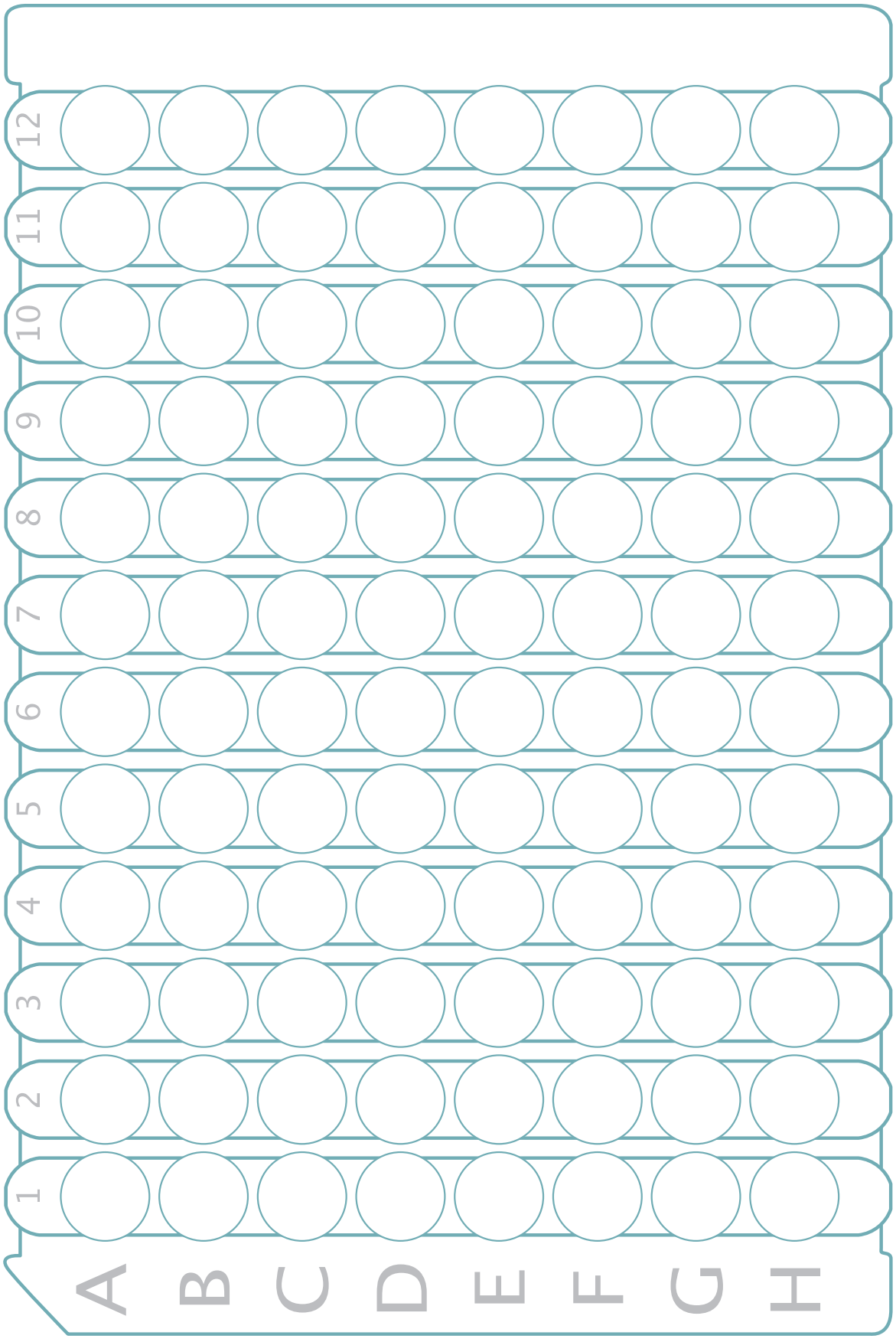
Recombinant mouse IGF-II interferes at concentrations ≥ 300 ng/mL in this assay.

REFERENCES

1. Schuller, A.G.P. *et al.* (1994) Mol. Cell. Endocrinol. **104**:57.
2. Ranke, M.B. and M. Elmlinger (1997) Horm. Res. **48**:9.
3. Butt, A.J. and A.C. Williams (2001) Apoptosis **6**:199.
4. Baxter, R.C. (2000) Am. J. Physiol. Endocrinol. Metab. **278**:E967.
5. Cohen, P. and R.G. Rosenfeld (1994) Curr. Opin. Pediatr. **6**:462.
6. Ali, O. *et al.* (2003) Horm. Metab. Res. **35**:726.
7. Hasegawa, T. *et al.* (1995) Growth Reg. **5**:36.
8. Schmid, C. *et al.* (2001) Eur. J. Endocrinol. **145**:213.
9. De Mellow, J.S.M. and R.C. Baxter (1988) Biophys. Res. Commun. **156**:199.
10. Moerman, E.J. *et al.* (1993) Exp. Gerontol. **28**:361.
11. Li, Y.M. *et al.* (1997) Endocrinology **138**:362.
12. Cohen, P. (2006) Endocrinology **147**:2109.
13. Chan, J.M. *et al.* (1998) Science **279**:563.
14. Chan, J.M. *et al.* (2002) J. Natl. Cancer Inst. **94**:1099.
15. Palmqvist, R. *et al.* (2002) Gut **60**:642.
16. Ma, J. *et al.* (1999) J. Natl. Cancer Inst. **91**:620.
17. Giovanucci, E. *et al.* (2000) Cancer Epidemiol. Biomarkers Prev. **9**:345.
18. Wakai, K. *et al.* (2002) Jpn. J. Cancer Res. **93**:1279.
19. Zhao, H. *et al.* (2003) J. Urol. **169**:714.
20. Petridou, E. *et al.* (2001) Oncology **60**:252.
21. Krajcik, R.A. *et al.* (2002) Cancer Epidemiol. Biomarkers Prev. **11**:1566.
22. Del Giudice, M.E. *et al.* (1998) Breast Cancer Res. Treat. **47**:111.
23. Gui, Y. and L.J. Murphy (2003) Mol. Cell. Biochem. **250**:189.
24. Huang, S.S. *et al.* (2003) FASEB J. **17**:2068.
25. Lee, K.W. *et al.* (2004) J. Biol. Chem. **279**:469.
26. Butt, A.J. *et al.* (2002) Endocrinology **143**:2693.
27. Liu, B. *et al.* (2000) J. Biol. Chem. **275**:33607.
28. Lee, K.W. *et al.* (2005) J. Biol. Chem. **280**:16942.
29. Ikonen, M. *et al.* (2003) Proc. Natl. Acad. Sci. USA **100**:13042.
30. Schedlich, L.J. *et al.* (2003) Endocrinology **144**:1984.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES