Quantikine® ELISA

Human Prolactin Immunoassay

Catalog Number DPRL00

For the quantitative determination of human Prolactin concentrations in 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
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	3
TECHNICAL HINTS	3
PRECAUTIONS	3
MATERIALS PROVIDED & STORAGE CONDITIONS	4
OTHER SUPPLIES REQUIRED	4
SAMPLE COLLECTION & STORAGE	5
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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INTRODUCTION

Prolactin (gene name PRL) is a secreted neuroendocrine pituitary hormone that acts primarily on the mammary gland to promote lactation, but has pleiotropic effects in both males and females (1-6). Prolactin is predominantly found as 199 amino acid, 25 kDa glycosylated and 23 kDa non-glycosylated monomers (7, 8). Human prolactin shares only 60% and 63% amino acid sequence identity with mouse and rat prolactin, respectively, although rat prolactin can activate the human prolactin receptor (3). Although better characterized in rodents than humans, post-translational modifications such as polymerization, glycosylation, and proteolytic cleavage can alter the activities of prolactin (7-10). Non-glycosylated prolactin is produced by the pituitary gland and packaged in storage granules before secretion. Glycosylated prolactin is reported to be constitutively secreted, have lower biological potency, and be removed from the circulation more quickly (3, 8, 9). Cleavage by Cathepsin D, or matrix metalloproteinases from chondrocytes, can produce N-terminal 16 kDa antiangiogenic fragments also called vasoinhibins (11-13). Alternatively, thrombin can produce C-terminal 16 kDa fragments that are not antiangiogenic (3). In humans, a large (>150 kDa) complex of prolactin with IgG that typically includes prolactin autoantibodies is termed macroprolactin (14, 15). Macroprolactin is cleared more slowly, thus contributing to high serum or plasma prolactin levels, and shows decreased prolactin bioactivity that is thought to be due to proximity of the IgG and receptor binding sites (15).

Prolactin is synthesized by the anterior pituitary in all mammals, with humans and rodents producing minor amounts in the decidua and lactating mammary gland (3). In humans, prolactin is also produced by the uterus, placenta, amnion, brain, prostate, dermal keratinocytes and fibroblasts, myometrium, stimulated leukocytes, adipocytes, and some breast cancer cells (2-6, 16-22). In the anterior pituitary, but not elsewhere, prolactin secretion is under tonic inhibition by hypothalamic dopamine (2, 3). Surges of prolactin secretion occur under the influence of estrogen and are influenced by light/dark cycles, especially in rodents (2, 3, 16). Prolactin expression is low during early human pregnancy, replaced by placental lactogen, but increases in late pregnancy (2, 3). It is present in plasma, milk, and amniotic fluid (3). The prolactin receptor (gene name PRLR) is a type I transmembrane glycoprotein that belongs to the cytokine hematopoietic receptor family. Expression of the prolactin receptor is widespread, including most leukocytes, $CD34^+$ progenitor cells, mammary gland, liver, kidney, adrenals, ovaries, testis, prostate, seminal vesicles, and hypothalamus (2-6, 23). Each prolactin molecule is thought to bind two receptor molecules (24). Splice forms with alternate sequences in the cytoplasmic portion of the human prolactin receptor have been found, are expressed in both normal and cancerous tissue, and may be strongly or weakly dominant-negative (25). A soluble splice variant lacking the transmembrane and cytoplasmic portion of the human prolactin receptor is predicted to bind prolactin, but appears to be rarely expressed *in vivo* (25).

INTRODUCTION *CONTINUED*

In addition to its lactogenic activity, peripherally produced prolactin plays roles in breast and prostate cancer development, regulation of reproductive function, and immunoregulation (5, 6, 15, 26). In human leukocytes, prolactin expression is reported to be activated by cAMP and activated or inhibited by several cytokines (3, 18-22). It may act as an autocrine or paracrine proliferative growth factor, and may enhance IFN-γ production and contribute to psoriasis (3-6, 17). Increases in serum or plasma prolactin (hyperprolactinemia) may or may not be due to macroprolactin. Macroprolactinemia is often asymptomatic, while hyperprolactinemia that does not involve macroprolactin may be associated with infertility, increased food intake, suppression of B cell tolerance, or suppression of anxiety in humans, and can occur due to benign pituitary prolactinomas or treatment with some antipsychotic medications (2, 4, 26). Removal of macroprolactin by polyethylene glycol (PEG) immunoprecipitation of immune complexes is thus an important method distinguishing hyperprolactinemia that is or is not due to macroprolactin (14). In general, prolactin measurement after PEG treatment will show <40% of the original prolactin value, while in the absence of macroprolactin >50% of prolactin will remain following PEG treatment (14). Treatment of hyperprolactinemia may include dopamine agonists to decrease pituitary prolactin production, or administration of antagonistic prolactin peptides (4, 27, 28). Diabetes may increase circulating prolactin and/or macroprolactin levels, and its cleaved vasoinhibin form may be protective against diabetic retinopathy (13, 29-31).

The Quantikine® Human Prolactin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Prolactin in serum and plasma. It contains *E. coli*-expressed recombinant human Prolactin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Prolactin 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 Prolactin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Prolactin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Prolactin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Prolactin 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 Prolactin 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.

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. Refer to the SDS on our website prior to use.

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 Prolactin Microplate	894372	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Prolactin.	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 Prolactin Conjugate	894373	21 mL of a polyclonal antibody specific for human Prolactin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Prolactin Standard	894374	Recombinant human Prolactin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6Z	895466	21 mL 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.
- Human Prolactin Controls (optional; R&D Systems®, Catalog # QC149).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at room temperature 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.*

REAGENT PREPARATION

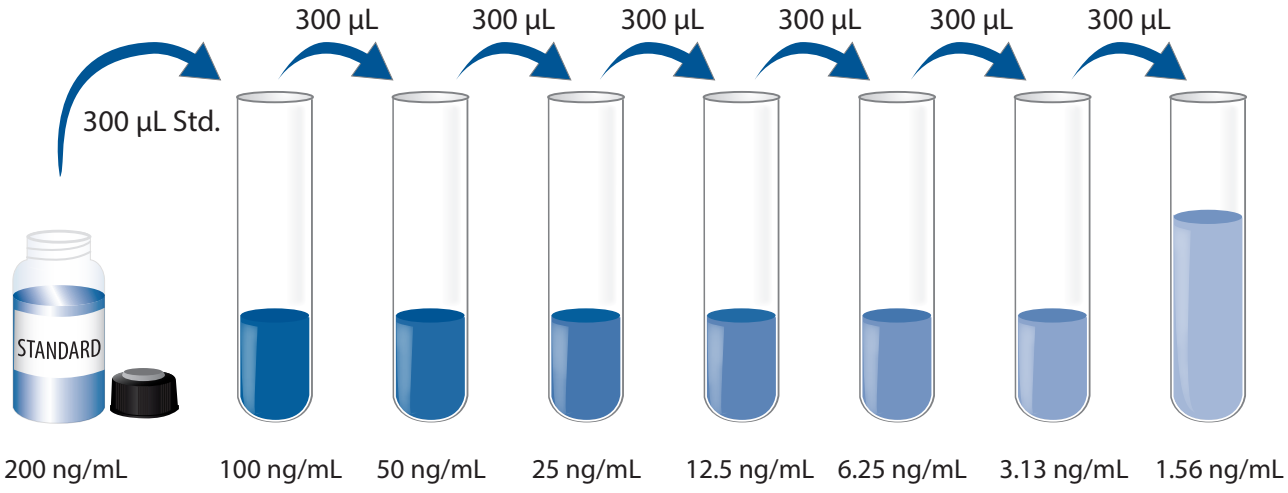
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 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 Prolactin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Prolactin 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 with gentle agitation prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD6Z into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 100 ng/mL standard serves as the high standard. Calibrator Diluent RD6Z 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 samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-19 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. 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 Prolactin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours 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.

CALCULATION OF RESULTS

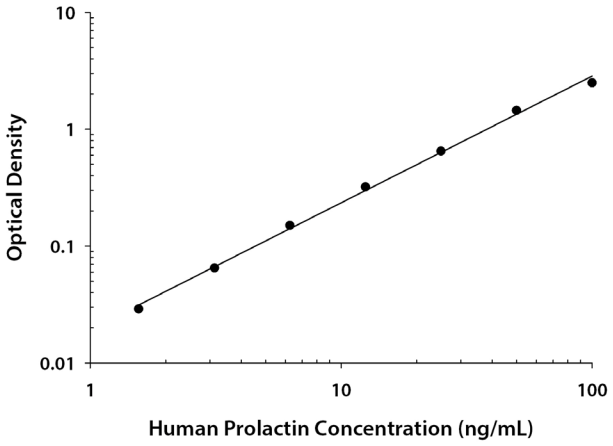
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Prolactin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been 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	0.009 0.009	0.009	—
1.56	0.037 0.038	0.038	0.029
3.13	0.071 0.077	0.074	0.065
6.25	0.159 0.159	0.159	0.150
12.5	0.329 0.331	0.330	0.321
25	0.653 0.662	0.658	0.649
50	1.442 1.471	1.457	1.448
100	2.419 2.585	2.502	2.493

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)	16.3	31.8	53.1	15.4	30.6	54.4
Standard deviation	0.831	1.24	1.79	0.90	1.48	2.94
CV (%)	5.1	3.9	3.4	5.8	4.8	5.4

RECOVERY

The recovery of human Prolactin spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Serum (n=4)	94	84-108%
EDTA plasma (n=4)	94	84-105%
Heparin plasma (n=4)	97	90-104%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human Prolactin were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	110	110	110
	Range (%)	106-115	105-116	108-113
1:4	Average % of Expected	107	109	109
	Range (%)	97-119	102-118	102-115
1:8	Average % of Expected	104	107	109
	Range (%)	94-116	96-114	100-119
1:16	Average % of Expected	101	101	102
	Range (%)	93-110	93-105	91-110

SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of human Prolactin ranged from 0.030-0.264 ng/mL. The mean MDD was 0.127 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 Prolactin manufactured by R&D Systems®. The NIBSC/WHO International Standard for Prolactin 84/500 was evaluated in this kit. The dose response curve of the International Standard parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® kit to approximate NIBSC (84/500) units, use the equation below.

NIBSC (84/500) approximate value (mIU/mL) = 0.0284 x Quantikine® Human Prolactin value (ng/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Prolactin 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=50)	17.7	3.00-106	15.9
EDTA plasma (n=50)	18.0	3.38-101	15.1
Heparin plasma (n=50)	17.9	3.45-108	16.1

Note: *Six of the fifty samples assayed measured above established prolactin ranges indicating these samples may contain macroprolactin. Macroprolactin can be removed from serum and plasma samples by using a PEG-precipitation protocol (14).*

SPECIFICITY

This assay recognizes natural and recombinant human Prolactin. This assay also recognizes natural human Macroprolactin.

The factors listed below were prepared at 200 ng/mL or 1000 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL or 1000 ng/mL in a mid-range recombinant human Prolactin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Chorionic Gonadotropin
Chorionic Gonadotropin α
Follicle-stimulating Hormone β
Growth Hormone
Luteinizing Hormone β
Thyroid-Stimulating Hormone β

Recombinant mouse:

Prolactin

Natural human proteins:

Albumin
Luteinizing Hormone β
Thyroid-Stimulating Hormone

Recombinant human Prolactin R interferes at concentrations > 25 ng/mL.

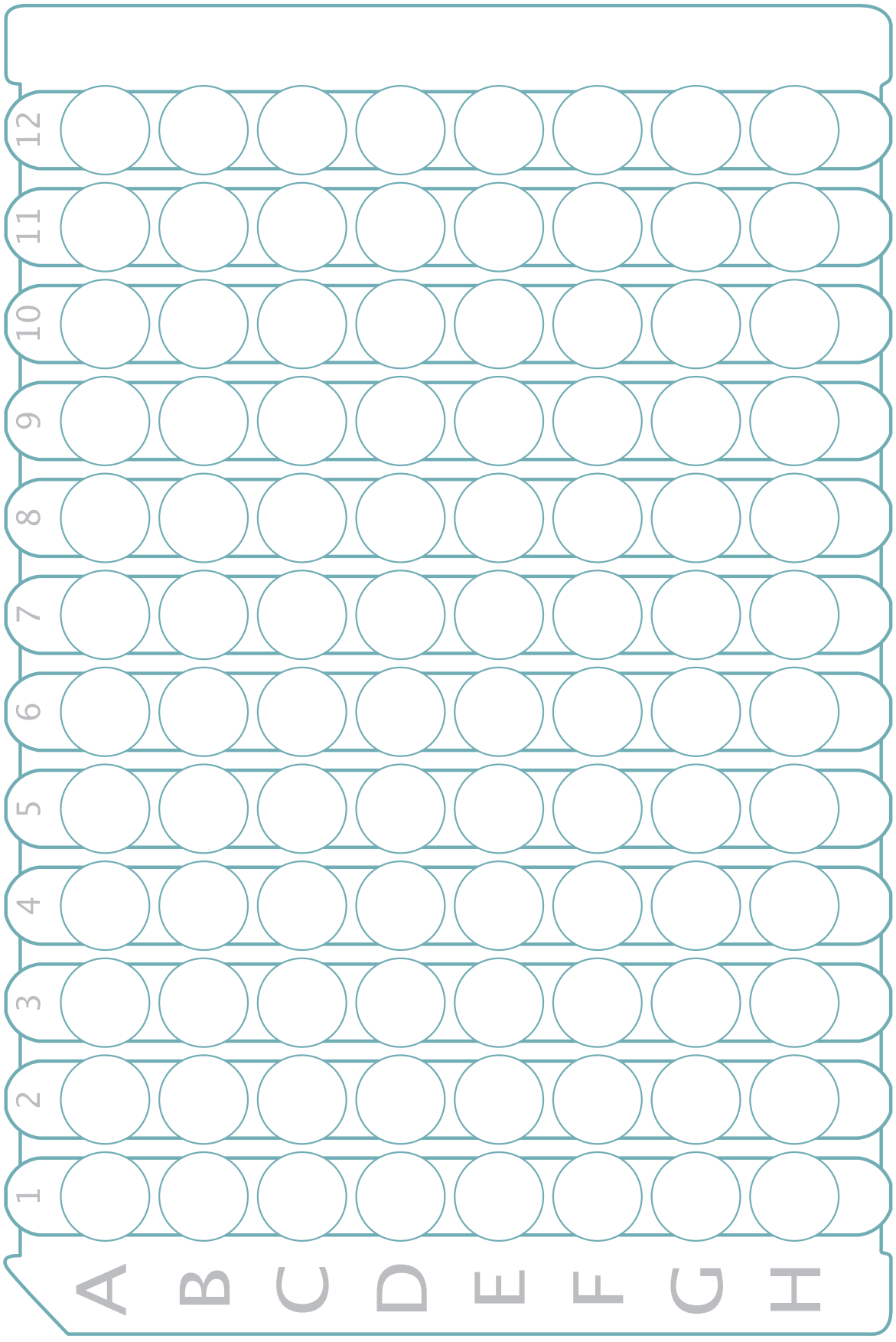
Recombinant mouse and rat Prolactin R interfere at concentrations > 6.25 ng/mL.

REFERENCES

1. Cooke, N.E. *et al.* (1981) J. Biol. Chem. **256**:4007.
2. Grattan, D.R. and I.C. Kokay (2008) J. Neuroendocrinol. **20**:752.
3. Ben-Jonathan, N. *et al.* (2008) Endocr. Rev. **29**:1.
4. Bernichtein, S. *et al.* (2010) J. Endocrinol. **206**:1.
5. Langan, E.A. *et al.* (2012) Arch. Dermatol. Res. **304**: 115.
6. Goffin, V. *et al.* (2011) Nat. Rev. Urology **8**:597.
7. Lewis, U.J. *et al.* (1985) Endoc. **116**:359.
8. Price, A.E. *et al.* (1995) Endoc. **136**:4827.
9. Hoffmann, T. *et al.* (1993) J. Endoc. Invest. **16**:807.
10. Cole, E. *et al.* (1991) Endoc. **129**:2639.
11. Piwnica, D. *et al.* (2006) Mol. Endocrinol. **20**:3263.
12. Macotela, Y. *et al.* (2006) J. Cell Sci. **119**:1790.
13. Triebel, J. *et al.* (2011) IUBMB Life **63**:806.
14. Olukoga, A.O. and J.W. Kane (1999) Clin. Endocrinol. (Oxf.) **5**:119.
15. Shimatsu, A. and N. Hattori (2012) Clin. Dev. Immunol. Dec. 4 [Epub ahead of print].
16. Featherstone, K. *et al.* (2012) J. Neuroendocrinol. **24**:977.
17. Langan, E.A. *et al.* (2010) Trends Endocrinol. Metab. **21**:569.
18. Semprini, S. *et al.* (2012) Endocrinology **153**:2724.
19. Gerlo, S. *et al.* (2006) J. Neuroimmunol. **172**:206.
20. Andria, M.L. and G.H. Reem (2007) Biochem. Biophys. Res. Commun. **354**:598.
21. Gerlo, S. *et al.* (2006) Cell Mol. Life Sci. **63**:92.
22. Gerlo, S. *et al.* (2005) J. Neuroimmunol. **162**:190.
23. Bellone, G. *et al.* (1995) J. Cell Physiol. **163**:221.
24. Broutin, I. *et al.* (2010) J. Biol. Chem. **285**:8422.
25. Trott, J. F. *et al.* (2003) J. Mol. Endocrinol. **30**:31.
26. Shelly, S. *et al.* (2012) Autoimmun. Rev. **11**:A465.
27. Jomain, J.B. *et al.* (2007) J. Biol. Chem. **282**:33118.
28. Bernichtein, S. *et al.* (2003) J. Biol. Chem. **278**:35988.
29. Arnold, E. *et al.* (2010) Diabetes **59**:3192.
30. Gulcelik, N.E. and A. Usman (2010) Neuro. Endocrinol. Lett. **31**:270.
31. Triebel, J. *et al.* (2009) Eur. J. Endocrinol. **161**:345.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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