Quantikine® QuicKit™ ELISA

Human Leptin Immunoassay

Catalog Number QK398

For the quantitative determination of human Leptin concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human Leptin (gene name OB) is a 16 kDa, 146 amino acid (aa) residue, non-glycosylated polypeptide that regulates adipose tissue mass and energy balance (1-6). The name Leptin is derived from the Greek (leptos, or "thin") because of its ability to reduce fat stores (7). In mice (ob/ob) and humans, inactivating mutations of the OB gene can cause obesity (1-6). Mature human Leptin shares 87% and 84% aa identity with mouse and rat Leptin, respectively (1, 8). Human Leptin is active in both the mouse and rat systems (9, 10). Leptin is expressed almost exclusively by adipocytes and its production is influenced by hormones, cytokines and nutrients (5, 8, 11). For example, Leptin expression is enhanced by insulin and glucocorticoids, which are associated with positive energy balance, while catecholamines decrease Leptin production during negative energy balance (5). It circulates in the plasma, crosses the blood-brain barrier, and is present in human breast milk (3-6, 12).

The human Leptin receptor (designated ObR or LEPR) is a 150 kDa, 1144 aa residue, type I transmembrane glycoprotein of the IL-6 receptor family of Class I cytokine receptors (13, 14). The gene for ObR undergoes considerable splicing, forming variants a-d with cytoplasmic domains of variable length, plus the potentially soluble form ObRe (14, 15). The long form, ObRb (formerly OB RL), is expressed mainly in the hypothalamic arcuate nucleus and is essential for signal transduction (6, 16, 17). Of the short forms, ObRa is ubiquitous, and ObRa, ObRc, and ObRd are all thought to mediate Leptin binding and endocytosis, but not signal transduction (16). Upon binding of Leptin dimers, ObRb dimers may form signaling tetramers with shorter forms (16). Mutations of ObRb can cause obese phenotypes in both the mouse and rat. The mouse mutation (db/db for diabetes) occurs in the cytoplasmic domain, while the rat mutation (fa/fa for fatty) occurs in the extracellular domain of the receptor (18, 19). In a concentration-dependent manner, Leptin signaling can have diverse effects, causing neurons that express pro-opiomelanocortin (POMC) peptides to reduce food intake, and neurons that express neuropeptide Y and agouti-related protein (NpY and AgRP) to increase food intake (4, 6).

Leptin is fundamentally a "starvation signal" that, when low, prompts increased appetite and decreased energy expenditure (4, 6, 10). Adipocytes increase Leptin expression as cell size increases, which should result in depressed appetite and increased energy expenditure (5). However, obese humans are often resistant to these effects of Leptin (3). Leptin resistance is in part due to saturation of the blood-brain transporter, which is influenced by high circulating triglycerides, and in part due to decreased cellular response to Leptin (6). Rarely, obese humans are genetically Leptin-deficient (3-6). Leptin deficiency also influences the immune system, depressing Th1 responses and causing increased frequency of infections (4). Leptin also regulates puberty, blocking the onset of puberty, or of menses if Leptin deficiency exists due to excessive thinness, such as results from starvation, extreme exercise-induced weight loss, anorexia or cancer-induced cachexia (3, 4).

The Quantikine® QuicKit™ Human Leptin Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human Leptin levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Leptin and antibodies raised against the recombinant protein. Results obtained using natural human Leptin showed linear curves that were parallel to the standard curves obtained using the QuicKit™ standards. These results indicate that this kit can be used to determine relative mass values for natural human Leptin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human Leptin. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of Leptin bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the 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® QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 Leptin Standard	899101	2 vials of recombinant human Leptin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human Leptin Capture Ab Concentrate	899099	Lyophilized tagged monoclonal antibody specific for human Leptin.	May be stored for up to 1 month at 2-8 °C.*
Human Leptin Detection Ab Concentrate	899100	400 µL of a monoclonal antibody specific for human Leptin conjugated to horseradish peroxidase with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a buffered protein base with preservatives. *Use diluted 1:10 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	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
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human Leptin Controls (optional; R&D Systems®, Catalog # QC264)

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.

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

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:10)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Calibrator Diluent RD5P (diluted 1:10) - Add 5.0 mL of Calibrator Diluent RD5P to 45 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:10).

Human Leptin Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Leptin Capture Ab Concentrate with Calibrator Diluent RD5P (diluted 1:10). This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

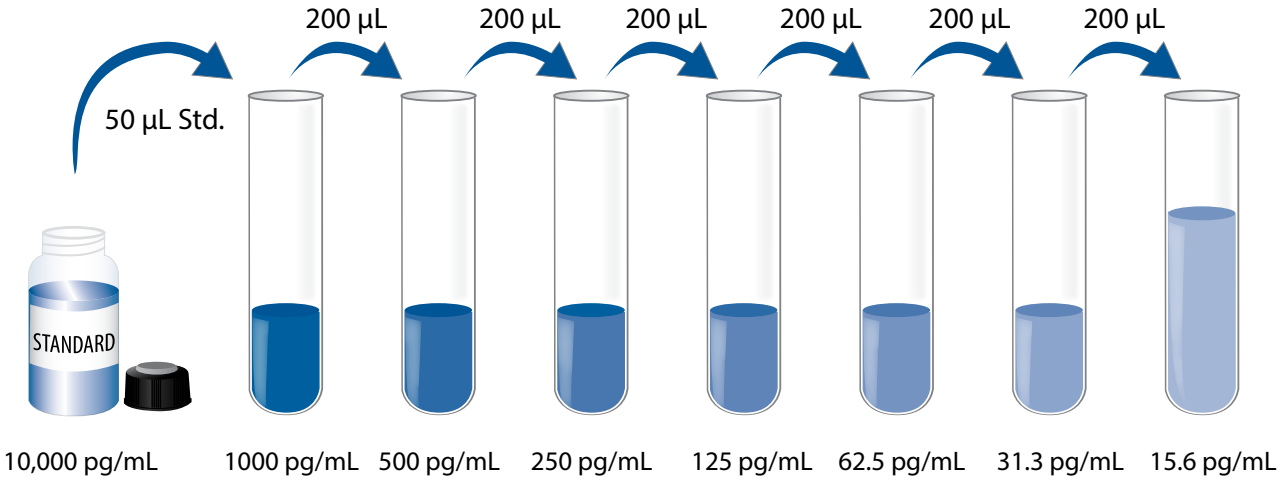
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Calibrator Diluent RD5P (diluted 1:10). For a full plate, add 300 µL of reconstituted Human Leptin Capture Ab stock and 300 µL of Human Leptin Detection Ab Concentrate to 5.4 mL of Calibrator Diluent RD5P (diluted 1:10).

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. 100 µL of the resultant mixture is required per well.

Human Leptin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Leptin Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:10) into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:10) 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, controls, and samples 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 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. 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.
8. 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

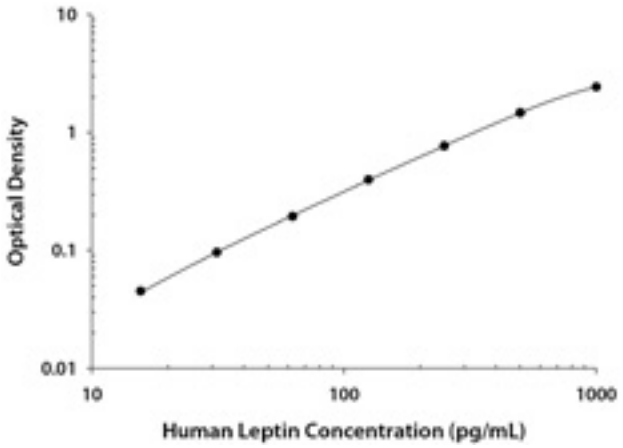
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Leptin 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	0.003 0.004	0.004	——
15.6	0.049 0.049	0.049	0.045
31.3	0.099 0.101	0.100	0.096
62.5	0.197 0.198	0.198	0.194
125	0.399 0.402	0.401	0.397
250	0.768 0.779	0.774	0.770
500	1.472 1.473	1.473	1.469
1000	2.427 2.450	2.439	2.435

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	134	709	130	709
Standard deviation	2.84	15.8	11.9	19.7
CV (%)	2.1	2.2	9.2	2.8

RECOVERY

The recovery of human Leptin spiked to three levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	89	80-106%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Leptin 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)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	102	107	104	104
	Range (%)	97-107	107-108	103-105	101-106
1:4	Average % of Expected	106	115	108	111
	Range (%)	99-119	113-118	107-108	106-115
1:8	Average % of Expected	113	121	113	115
	Range (%)	103-128	118-124	111-114	110-121
1:16	Average % of Expected	117	125	113	118
	Range (%)	112-129	117-133	107-119	112-124

SENSITIVITY

Ten assays were evaluated and the minimum detectable dose (MDD) of human Leptin ranged from 0.292-1.03 pg/mL. The mean MDD was 0.538 pg/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 Leptin produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type		Range (pg/mL)	Mean (pg/mL)	% Detectable
Male	Serum (n = 10)	ND-10,671	7144	70
	EDTA plasma (n=10)	ND-10,882	6246	80
	Heparin plasma (n=10)	ND-8691	6034	70
Female	Serum (n = 10)	7682-79,571	36,063	100
	EDTA plasma (n=10)	7669-76,640	36,323	100
	Heparin plasma (n=10)	5335-77,190	34,210	100

ND=Non-detectable

Cell Culture Supernates - Human primary subcutaneous pre-adipocytes were collected before differentiation or differentiated into adipocytes using commercially available media from Zen-Bio. Aliquots of the cell culture supernates were removed on days 0, 7, 13, 21, and 28 and assayed for levels of human Leptin.

Day of Adipocyte Differentiation	(pg/mL)
0	ND
7	319
13	1684
21	4952
28	7551

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human Leptin.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level recombinant human Leptin control were assayed for interference. No significant cross-reactivity or interference was observed.

Other recombinant:

mouse Leptin
rat Leptin

Recombinant human Leptin R Fc chimera does not cross-react but does interfere at concentrations ≥ 2.0 ng/mL in this assay.

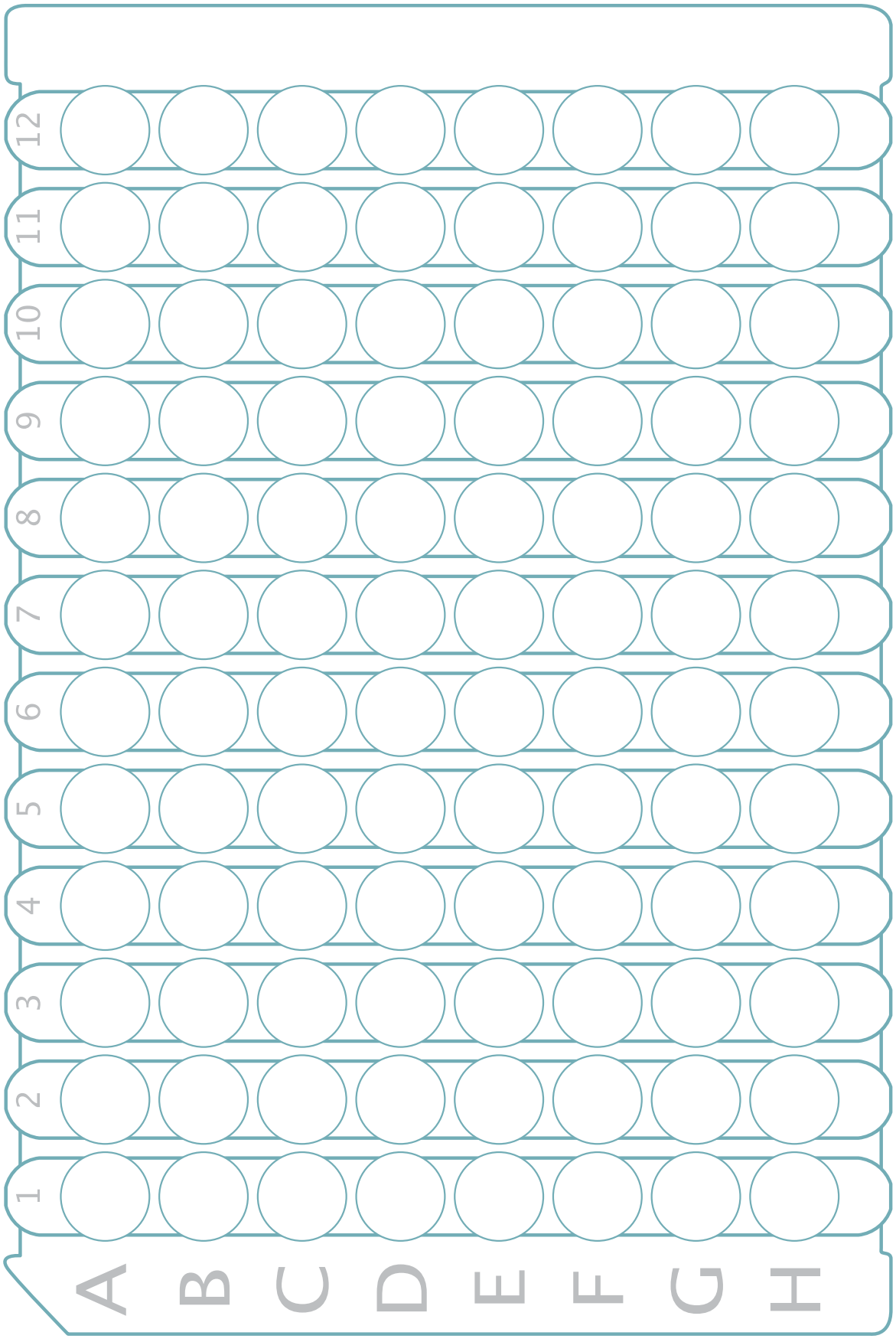
Recombinant mouse Leptin R Fc chimera does not cross-react but does interfere at concentrations ≥ 1.0 ng/mL in this assay.

REFERENCES

1. Zhang, Y. *et al.* (1994) Nature **372**:425.
2. Cohen, S.L. *et al.* (1996) Nature **382**:589.
3. Friedman, J.M. (2009) Am. J. Clin. Nutr. **89**:973S.
4. Farooqi, I.S. and S. O'Rahilly (2009) Am. J. Clin. Nutr. **89**:980S.
5. Lee, M-J. and S.K. Fried (2009) Am. J. Physiol. Endocrinol. Metab. **296**:E1230.
6. Oswal, A. and G. Yeo (2010) Obesity **18**:221.
7. Halaas, J.L. *et al.* (1995) Science **269**:543.
8. Ogawa, Y. *et al.* (1995) J. Clin. Invest. **96**:1647.
9. Verploegen, S.A.B.W. *et al.* (1997) FEBS Lett. **405**:237.
10. Satoh, N. *et al.* (1997) Neurosci. Lett. **224**:149.
11. Leroy, P. *et al.* (1996) J. Biol. Chem. **271**:2365.
12. Savino, F. *et al.* (2010) Eur. J. Clin. Nutr. **64**:972.
13. Cohen, B. *et al.* (1996) Science **274**:1185.
14. Tartaglia, L.A. *et al.* (1995) Cell **83**:1263.
15. Murakami, T. *et al.* (1997) Biochem. Biophys. Res. Commun. **231**:26.
16. Bacart, J. *et al.* (2010) FEBS Lett. **584**:2213.
17. Tu, H. *et al.* (2007) J. Cell. Physiol. **212**:215.
18. Chen, H. *et al.* (1996) Cell **84**:491.
19. Phillips, M.S. *et al.* (1996) Nat. Genet. **13**:18.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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