DRG® Leptin (Rat) ELISA (EIA-4607)

Revised 2 Feb. 2011 rm (Vers. 2.1) **FOR RESEARCH USE ONLY**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

This kit is not intended for diagnostic purposes.

1 INTENDED USE

This kit is used for the non-radioactive determination of leptin in rat sera.

Plasma samples may also be used but application to samples of other biological fluids may need validation by the user. One kit is sufficient to measure 37 unknown samples in duplicate.

This kit is for research purpose only.

2 PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) binding of leptin in the sample by a pre-titered antiserum and immobilization of the resulting complexes in the wells of a microtiter plate, 2) after washing purified biotinylated detection antibody is allowed to bind to the immobilized leptin, 3) binding of horseradish peroxidase to the immobilized biotinylated antibodies after free detection antibodies are washed off, 4) wash away of free enzyme conjugates, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590nm, after acidification of formed products.

Since the increase in absorbency is directly proportional to the amount of captured leptin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of rat leptin.

3 REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

A. **Rat/Mouse Leptin ELISA Plate;** Coated with pre-titered capture antibodies
Quantity: 1 plate
Preparation: Ready to use.
Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2 °C - 8 °C.

B. **Adhesive Plate Sealer;**
Quantity: 2 sheets
Preparation: Ready to use.

C. **Rat/Mouse Leptin Antiserum;** Pre-titered anti-rodent leptin serum
Quantity: 6 mL
Preparation: Ready to use.

D. **10X HRP Wash Buffer Concentrate**
10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.
Quantity: Two bottles containing 50 mL each
Preparation: Dilute 1:10 with distilled or de-ionized water.

E. **Rat Leptin Standards;** Rat leptin in buffer: 0.2, 0.5, 1, 2, 5, 10, 20 and 30 ng/mL.
Quantity: 0.5 mL/vial
Preparation: Ready to use.

F. **Quality Controls 1 and 2;** Various peptides including leptin in QC buffer.
Quantity: 0.5 mL/vial
Preparation: Ready to use.

G. **Rat/Mouse Leptin Matrix Solution;** Matrix containing 0.08% Sodium Azide
Quantity: 0.5 mL/vial
Preparation: Ready to use.

H. **Assay Buffer;** 0.05 M phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.08% sodium azide, 0.05% Triton X-100 and 1% BSA.
Quantity: 20 mL/vial
Preparation: Ready to use.

I. **Rat/Mouse Leptin Detection Antibody;** Pre-titered biotinylated anti-mouse leptin antibody.
Quantity: 12 mL/vial
Preparation: Ready to use.

J. **Enzyme Solution;** Pre-titered streptavidin-horseradish peroxidase conjugate in buffer.
Quantity: 12 mL/vial
Preparation: Ready to use.

K. **Substrate (Light Sensitive: avoid unnecessary exposure to light)**
3,3',5,5'-tetramethylbenzidine in buffer.
Quantity: 12 mL/vial
Preparation: Ready to use.

L. **Stop Solution (Caution: Corrosive Solution);** 0.3 M HCl
Quantity: 12 mL/vial
Preparation: Ready to use.

4 STORAGE AND STABILITY

All components of the kit can be stored up to two weeks at 2 °C - 8 °C. For longer storage (>2 weeks), freeze antiserum, standards, quality controls, and matrix solution at –20 °C and avoid repeated freeze and thaw. Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2 °C - 8 °C.

Refer to expiration dates on all reagents prior to use.

Do not mix reagents from different kits unless they have the same lot numbers.

5 REAGENT PRECAUTIONS

A. **Sodium Azide**

Sodium azide has been added to certain reagents as a preservative at a concentration of 0.08 %. Although it is at a minimum concentration, sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

B. **Hydrochloric Acid**

Hydrochloric acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eye. Do not swallow or ingest.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and pipette tips: 10 µL – 20 µL and 20 – 100 µL
2. Multi-channel Pipettes and pipette tips: 0 – 50 µL and 50 – 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. De-ionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

7 SAMPLE COLLECTION AND STORAGE

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 °C ± 2 °C.
3. Transfer and store serum samples in separate tubes. Date and identify each sample.
4. Use freshly prepared serum or aliquot and store samples at ≤ –20°C for later use.
   Avoid multiple (> 3) freeze/thaw cycles.
5. To prepare plasma sample, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/ml and centrifuge immediately after collection. Observe the same precautions in the preparation of serum samples.
6. If heparin is to be used as anti-coagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
7. Avoid using samples with gross hemolysis or lipemia.

8 ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay.

1. Dilute the 10X concentrated HRP Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or distilled water.
2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2 °C - 8 °C. Assemble strips in an empty plate holder and wash each well 3 times with 300 µL of diluted Wash Buffer per wash. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If automated machine is used for assay, follow the manufacturer's instructions for all washing steps described in this protocol.**
3. Add 30 µL Assay Buffer to Background wells, Standard wells, and QC1 and QC2 wells.
   Add 40 µL Assay Buffer to sample wells.
4. If samples to be assayed are serum or plasma, add 10 µL Matrix Solution to the Background wells, Standard wells, and QC1 and QC2 wells. If samples are free of significant serum matrix components, add 10 µL Assay Buffer instead.
5. Add 10 µL Assay Buffer to the Background wells and add in duplicates 10 µL Rat Leptin Standards in the order of ascending concentration to the appropriate wells.
6. Add 10 µL QC1 and 10 µL QC2 to the appropriate wells.
7. Add sequentially 10 µL of the unknown samples in duplicate to the remaining wells.
8. Transfer Antiserum Solution to a reagent reservoir and add 50 µL of this solution to each well with a multi-channel pipette. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.
9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.
10. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
11. Add 100 µL Detection Antibody to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-5 00 rpm.
12. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.
13. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
14. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the micro-titer plate shaker.
15. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.
16. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

17. Add 100 µL of Substrate solution to each well, cover plate with sealer and shake in the plate shaker for approximately 10 to 15 minutes. Blue color should be formed in wells of Leptin standards with intensity proportional to increasing concentrations of Leptin.

    NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time. One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.

18. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn into yellow after acidification. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there is no air bubbles in any well. Record the difference of absorbance units.

Assay Procedure for Rat Leptin ELISA

	Well #	Step 1	Step 2	Step 3	Step 4	Step 5-7	Step 8	Step 9-10	Step 11	Step 12-13	Step 14	Step 15-16	Step 17		Step 18	
		Dilute both bottles of 10X Wash Buffer with 900mL Deionized Water.	Wash plate 3X with 300 µl Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	Assay Buffer	Matrix*	Standards/ Controls/ Samples	Antiserum	Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer	Detection Antibody	Seal, Agitate, Incubate 1 hour at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels. Wash 3X with 300 µL Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µl Wash Buffer	Substrate	Seal, Agitate, Incubate 10-15 minutes at Room Temperature.	Stop Solution	Shake by hand. Read Absorbance at 450 nm and 590 nm within 5 minutes.
	A1, B1			40 µL	10 µL	--------	50 µL		100 µL		100 µL		100 µL		100 µL	
	C1, D1			30 µL	10 µL	10 µL of 0.2 ng/mL Standard	50 µL									
	E1, F1			30 µL	10 µL	10 µL of 0.5 ng/mL Standard	50 µL									
	G1, H1			30 µL	10 µL	10 µL of 1 ng/mL Standard	50 µL									
	A2, B2			30 µL	10 µL	10 µL of 2 ng/mL Standard	50 µL									
	C2, D2			30 µL	10 µL	10 µL of 5 ng/mL Standard	50 µL									
	E2, F2			30 µL	10 µL	10 µL of 10 ng/mL Standard	50 µL									
	G2, H2			30 µL	10 µL	10 µL of 20 ng/mL Standard	50 µL									
	A3, B3			30 µL	10 µL	10 µL of 30 ng/mL Standard	50 µL									
	C3, D3			30 µL	10 µL	10 µL of QC 1	50 µL									
	E3, F3			30 µL	10 µL	10 µL of QC 2	50 µL									
	G3, H3			40 µL	--------	10 µL of Sample	50 µL									
	A4, B4...			40 µL	--------	10 µL of Sample	50 µL									

* See Section 8. Assay Procedure Step 4:
If samples are free of significant matrix components, add 10 µL Assay buffer instead.

9 MICROTITER PLATE ARRANGEMENT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	2.0 ng/mL	30 ng/mL	Sample 2								
B	Blank	2.0 ng/mL	30 ng/mL	Sample 2								
C	0.2 ng/mL	5.0 ng/mL	QC 1	Etc.								
D	0.2 ng/mL	5.0 ng/mL	QC 1									
E	0.5 ng/mL	10 ng/mL	QC 2									
F	0.5 ng/mL	10 ng/mL	QC 2									
G	1.0 ng/mL	20 ng/mL	Sample 1									
H	1.0 ng/mL	20 ng/mL	Sample 1									

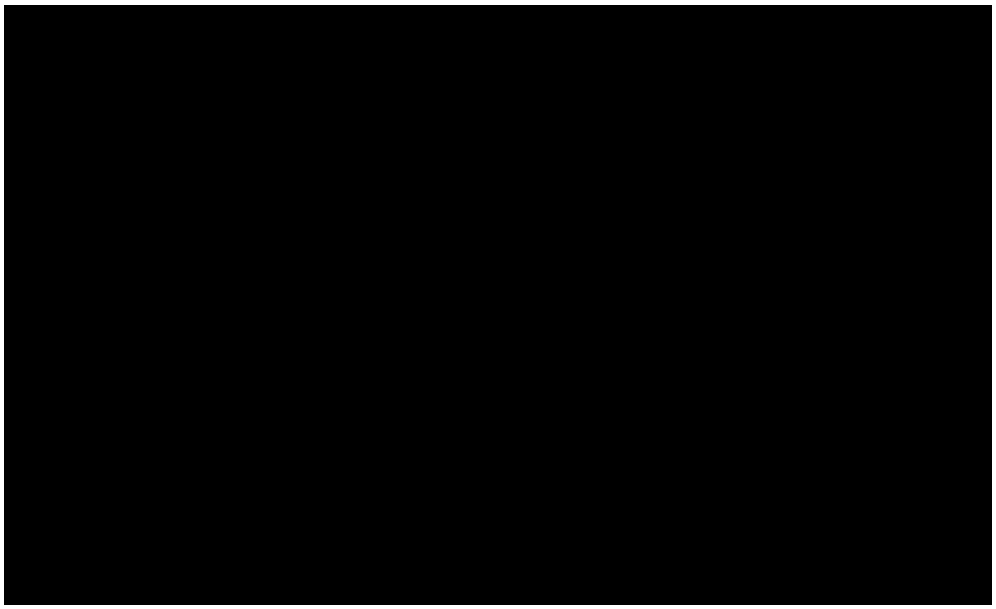
10 CALCULATIONS

Graph a reference curve by plotting the absorbance unit of 450 nm, less unit at 590 nm, on the Y-axis against the concentrations of rat leptin standard on the X-axis. The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function.

[**Note:** When sample volumes assayed differ from 10 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 5 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µL, compensate the volume deficit with either matrix solution or assay buffer, whichever is appropriate.

11 GRAPH OF TYPICAL REFERENCE CURVE

(n = 9 assays)



0.1 1 10 100

Rat Leptin, ng/mL

For Demonstration Only - Do not use for calculations

12 CORRELATION GRAPH

Rat Leptin Assays:

Correlation of Results by RIA and ELISA Methods

Serum samples from 27 rats were assayed for leptin using both DRG Rat Leptin RIA Kit (Cat#: RIA-1814) and Rat Leptin ELISA Kit (Cat#: EIA-4607). Correlation of the two kits is derived by linear regression analysis of paired results from each sample.

$y = 0.696\,x + 0.305$

$r = 0.993$

ORDERING INFORMATION

Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for research use only.

Material Safety Data Sheets (MSDS)

Material safety data sheets for the product may be ordered by fax or phone.