DRG® Adiponectin (Rat) ELISA (EIA-4570)

Revised 10 Sept. 2010 rm (Vers. 2.1) **For Veterinary Use Only**

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

1 INTENDED USE

This kit is for research purpose only.

2 PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of Adiponectin molecules from samples to the wells of a microtiter plate coated with a monoclonal anti-adiponectin antibody, 2) washing of unbound materials from samples, 3) binding of a second biotinylated monoclonal anti-adiponectin antibody to the captured molecules , 4) washing of unbound materials from samples, 5) binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibody, 6) washing of excess free enzyme conjugates, and 7) 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 absorbance at 450 nm – 590 nm after acidification of formed products.

Since the increase in absorbance is directly proportional to the amount of captured Rat Adiponectin 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 Adiponectin.

3 REAGENTS SUPPLIED

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

A. **Rat Adiponectin ELISA Plate**
Coated with Monoclonal Anti-Adiponectin 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 2-8°C.

B. **Adhesive Plate Sealer**
Quantity: 21 sheets
Preparation: Ready to Use

C. **10X HRP Wash Buffer Concentrate**
10X concentrate of 50 mM Tris Buffered Saline containing Tween-20
Quantity: 2 bottles containing 50 ml each
Preparation: Dilute 1:10 with distilled or deionized water

D. **Rat Adiponectin Standard**
Adiponectin Calibrator lyophilized.
Quantity: 200 ng/ml upon hydration.
Preparation: Contents Lyophilized.
Reconstitute with 0.5 ml distilled or deionized water to obtain 200 ng/ml.

E. **Rat Adiponectin Quality Controls 1 and 2**
One vial each, lyophilized, containing diluted serum at two different levels of Adiponectin.
Quantity: 0.5ml/vialbottle upon hydration
Preparation: Contents Lyophilized.
Reconstitute each vial with 0.5ml distilled or deionized water

F. **10X Assay Buffer (Sample Diluent)**
Quantity: 50 ml
Preparation: Dilute 1:10 with distilled or deionized water to make 1X Assay Buffer
(0.05M Phosphosaline containing 0.025M EDTA, 0.08% Sodium Azide, 1% BSA)
Note: Use 1X Assay Buffer to dilute samples (Section SAMPLE PREPARATION) and Standard Curve (Section STANDARD AND QUALITY CONTROLS PREPARATION)

G. **Assay RunningBuffer**
0.05M Phosphosaline containing 0.025M EDTA, 0.08% Sodium Azide, 1% BSA, and animal serum IgG
Quantity: 10 ml
Preparation: Ready to Use

H. **Rat Adiponectin Detection Antibody**
Pre-titered Biotinylated Monoclonal anti-Adiponectin Antibody
Quantity: 12 ml
Preparation: Ready to Use

I. **Enzyme Solution**
Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 ml
Preparation: Ready to Use

J. **Substrate (**Light sensitive, avoid unnecessary exposure to light)
3,3´,5,5´-tetramethylbenzidine in buffer
Quantity: 12 ml
Preparation: Ready to Use.

K. **Stop Solution** (Caution: Corrosive Solution)
0.3 M HCl
Quantity: 12 ml
Preparation: Ready to Use

4 STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C.

For longer storage (> 2 weeks), freeze diluted Wash Buffer, Assay Buffer, and reconstituted Standards and Controls at ≤ –20°C.

Minimize repeated freeze and thaw of the Adiponectin Standards and Quality Controls.

Unused microtiter strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°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. Although the concentrations are low, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a 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 eyes. Do not swallow or ingest.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µL and 50 ~ 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 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 ± 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. For long-term storage, keep at -70 °C. Avoid freeze/thaw cycles.
5. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/ml and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
6. If heparin is to be used as an anticoagulant, 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 SAMPLE PREPARATION

1. Allow all the reagents to come to room temperature.
2. Dilute serum or plasma samples **1:500** in 1X Assay Buffer (Sample Diluent).
   Cellular extract and culture media dilutions will vary.
3. Make Dilution A with 10 µl sample to 990 µl of 1X Assay Buffer (Sample Diluent) and mix well.
4. Make Dilution B by adding 100 µl of Dilution A to 400 µl of 1X Assay Buffer (Sample Diluent) and mixing well.
   Use Dilution B (1:500) for the assay procedure.

9 STANDARD AND QUALITY CONTROLS PREPARATION

A. Rat Adiponectin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using an Eppendorf pipette, reconstitute the Rat Adiponectin Standard with 0.5 ml distilled or deionized water into the glass vial to give a 200 ng/mL concentration of Standard. Invert and mix gently, let sit for 5 minutes then vortex gently.
2. Label six tubes 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml.
   Add 0.25 ml of 1X diluted Assay Buffer (Sample Diluent) to each of the six tubes.
   Prepare serial dilutions by adding 0.25 ml of the 200 ng/ml reconstituted standard to the 100 ng/ml tube, mix well and transfer 0.25 ml of the 100 ng/ml standard to the 50 ng/ml tube,
   mix well and transfer 0.25 ml of the 50 ng/ml Standard to the 25 ng/ml tube,
   mix well and transfer 0.25 ml of the 25 ng/ml Standard to the 12.5 ng/ml tube,
   mix well and transfer 0.25 ml of the 12.5 ng/ml Standard to the 6.25 ng/ml tube,
   mix well and transfer 0.25 ml of the 6.25 ng/ml Standard to the 3.125 ng/ml tube and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ –20°C. Avoid multiple freeze/thaw cycles.

Standard Concentration ng/ml	Volume of Deionized Water to Add	Volume of Standard to Add
200	0.5 ml	0
Standard Concentration ng/ml	**Volume of 1X diluted Assay Buffer (Samples Diluent) to Add**	**Volume of Standard to Add**
100	0.25 ml	0.25 ml of 200 ng/ml
50	0.25 ml	0.25 ml of 100 ng/ml
25	0.25 ml	0.25 ml of 50 ng/ml
12.5	0.25 ml	0.25 ml of 25 ng/ml
6.25	0.25 ml	0.25 ml of 12.5 ng/ml
3.125	0.25 ml	0.25 ml of 6.25 ng/ml

B. Rat Adiponectin Quality Control 1 and 2 Preparation

1. Use care in opening the lyophilized Quality Control vials.
   Using an Eppendorf pipette, reconstitute each of the Rat Adiponectin Quality Control 1 and Quality Control 2 with 0.5 ml distilled or deionized water into the glass vials.
   Invert and mix gently, let sit for 5 minutes then mix well.

10 ASSAY PROCEDURE

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

1. Dilute the 10X Wash Buffer concentrate 10 fold by mixing the entire contents of each both bottles of Wash Buffer with 900 ml deionized 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 2-8°C. Assemble the 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 an automated machine is used for the assay, follow the manufacturer´s instructions for all washing steps described in this protocol.
3. Add 80 µL **Assay Running Buffer** to all wells.
4. Add in duplicate 20 µL **Assay Running Buffer** to blank wells.
5. Add in duplicate 20 µL Rat Adiponectin Standards in the order of ascending concentration to the appropriate wells.
   Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells.
   Add sequentially 20 µL of the unknown samples in duplicate to the remaining wells.
   (See plate well map for suggested well orientation).
   For best result all additions should be completed within 30 minutes. 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, approximately 400 to 500 rpm.
6. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
7. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
8. Add 100 µL Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
10. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
11. 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 microtiter plate shaker.
12. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

13. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
14. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 25 minutes. Blue color should be formed in wells of the Rat Adiponectin standards with intensity proportional to increasing concentrations of Rat Adiponectin.

    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 a 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.
15. 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 to yellow after acidification. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of highest Adiponectin standard should be approximately 2.2-2.8, or not to exceed the capability of the plate reader used.

Assay Procedure

	Well #	Step 1	Step 2	Step 3-4	Step 5	Step 6-7	Step 8	Step 9-10	Step 11	Step 12-13	Step 14	Step 14	Step 15	Step 15
	A1, B1	Dilute both bottles of 10X Wash Buffer with 900 ml Deionized Water.	Wash plate 3X with 300 µl Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	Assay Running Buffer 100 µl	Standards / Controls / Samples —	Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µl Wash Buffer	Detection Antibody 100 µl	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 100 µl	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 3X with 300 µl Wash Buffer	Substrate 100 µl	Seal, Agitate, Incubate 5-25 minutes at Room Temperature.	Stop Solution 100 µl	Read Absorbance at 450 nm and 590 nm.
	C1, D1			80 µl	20 µl of 3.125 ng/ml Standard									
	E1, F1			80 µl	20 µl of 6.25 ng/ml Standard									
	G1, H1			80 µl	20 µl of 12.5 ng/ml Standard									
	A2, B2			80 µl	20 µl of 25 ng/ml Standard									
	C2, D2			80 µl	20 µl of 50 ng/ml Standard									
	E2, F2			80 µl	20 µl of 100 ng/ml Standard									
	G2, H2			80 µl	20 µl of 200 ng/ml Standard									
	A3, B3			80 µl	20 µl of QC 1									
	C3, D3			80 µl	20 µl of QC 2									
	E3, F3			80 µl	20 µl of Sample									
	G3, H3 →			80 µl	20 µl of Sample									

11 MICROTITER PLATE ARRANGEMENT

Adiponectin Rat ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	25 ng/mL	QC 1	Etc.								
B	Blank	25 ng/mL	QC 1									
C	3.125 ng/mL	50 ng/mL	QC 2									
D	3.125 ng/mL	50 ng/mL	QC 2									
E	6.25 ng/mL	100 ng/mL	Sample 1									
F	6.25 ng/mL	100 ng/mL	Sample 1									
G	12.5 ng/mL	200 ng/mL	Sample 2									
H	12.5 ng/mL	200 ng/mL	Sample 2									

12 CALCULATIONS

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.

Final results should be multiplied by a 500 dilution factor.

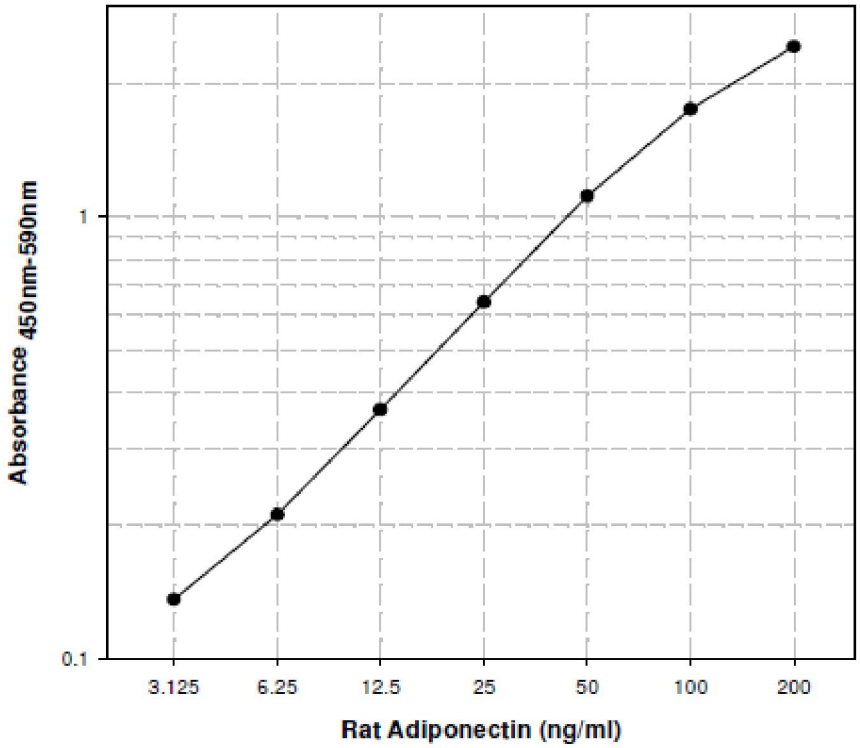
Note: When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µl, compensate the volume deficit with assay buffer (sample diluent).

13 INTERPRETATION

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC´s fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.

3. The limit of sensitivity of this assay is 0.155 ng/ml Rat Adiponectin (20 µL sample size).
4. The appropriate range of this assay is 3.125 ng/ml to 200 ng/ml Rat Adiponectin (20 µL sample size). Any result greater than 200 ng/ml in a 20 µL sample should be diluted using sample diluent, and the assay repeated until the results fall within range.

14 STANDARD CURVE



Typical Standard Curve, not to be used to calculate data.

15 ASSAY CHARACTERISTICS

15.1 Sensitivity

The lowest level of Adiponectin that can be detected by this assay is 0.155 ng/ml when using a 20 µL sample size.

15.2 Specificity

The antibody pair used in this assay does not cross-react with mouse adiponectin and other rat, mouse and human cytokine or hormone molecules tested.

Analyte	Max. Concentration	Cross-Reactivity
Human Resistin	20 ng/ml	n.d
Human ASP	1000 ng/ml	n.d
Mouse Adiponectin	100 ng/ml	n.d
Rat Cytokines:	40 ng/ml*	n.d.
IL-1α, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-17, IL-18, IP-10, GRO/KC, TNF-α, IFN-γ, G-CSF, MCP-1, Eotaxin, Leptin, MIP-1α, RANTES, VEGF, and Resistin. *Rat Leptin Max Conc. = 200 ng/ml *Rat Resistin Max Conc. = 100 ng/ml		

n.d. = not detectable

15.3 Precision

Intra- Assay Variation

Sample No.	Mean Adiponectin Levels (ng/ml)	Intra- % CV
1	12.04	0.43
2	16.44	1.52
3	17.61	0.98
4	17.81	1.18
5	19.45	0.82
6	20.4	1.96
7	21.8	1.73
8	33.0	1.59

The intra-assay variations of DRG Adiponectin Rat ELISA kits were studied on six rat serum samples and two rat plasma samples with varying concentrations of endogenous Adiponectin.

The mean intra-assay variation was calculated from results of 4 duplicate determinations in each assay of the indicated samples.

Inter-Assay Variation

Sample No.	Mean Adiponectin Levels (ng/ml)	Inter- % CV
1	11.05	7.84
2	15.35	7.3
3	16.18	7.3
4	16.38	7.5
5	18.05	6.41
6	19.58	4.3
7	20.68	8.44
8	30.68	6.54

The inter-assay variations of DRG Adiponectin Rat ELISA kits were studied on six rat serum samples and two rat plasma samples with varying concentrations of endogenous Adiponectin.

The mean inter-assay variations of each sample was calculated from results of four separate assays with duplicate samples in each assay.

15.4 Recovery

Spike & Recovery of Rat Adiponectin in Serum

Sample No.	Adiponectin Added ng/ml	Expected ng/ml	Observed ng/ml	% of Recovery
1	0	24.9	24.9	100
	6.25	31.15	31.6	101
	25.0	49.9	48.2	97
	100.0	124.9	112.9	90
2	0	29.5	29.5	100
	6.25	35.75	36.5	102
	25.0	54.5	55.4	102
	100.0	129.5	117.2	91
3	0	19.6	19.6	100
	6.25	25.85	26.8	104
	25.0	44.6	44.5	100
	100.0	129.5	106.6	82
4	0	20.1	20.1	100
	6.25	26.35	27	102
	25.0	45.1	45.2	100
	100.0	120.1	111	92

Varying amounts of Rat Adiponectin were added to four Rat serum samples and the Adiponectin content was determined in three separate assays.

The % of recovery = observed Adiponectin concentrations/expected Adiponectin concentrations x 100%.

15.5 Linearity

Effect of Serum Dilution

Sample No.	Volume Sampled	Expected ng/ml	Observed ng/ml	% Of Expected
1	20 µl	25	25	100
	10 µl	12.5	12.5	100
	5 µl	6.25	6.1	98
	2.5 µl	3.125	3.1	99
2	20 µl	30.9	30.9	100
	10 µl	15.45	16.1	104
	5 µl	7.725	8.2	106
	2.5 µl	3.863	4.2	109
3	20 µl	20.1	20.1	100
	10 µl	10.05	10.5	104
	5 µl	5.025	5.6	111
	2.5 µl	2.513	2.6	103
4	20 µl	20	20	100
	10 µl	10	10.5	105
	5 µl	5	5.9	118
	2.5 µl	2.5	3	120

Four Rat serum samples with the indicated sample volumes were assayed in three separate experiments. Required amounts of assay buffer were added to compensate for lost volumes below 20 µl. The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 20 µl, 10 µl, 5 µl, and 2.5 µl sample volumes assayed, respectively, were applied in the calculation of observed Adiponectin concentrations.

% expected = observed/expected x 100%.

16 QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert

17 TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

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 in vitro use only.

Material Safety Data Sheets (MSDS)

Material safety data sheet for this product may be ordered by fax or phone.