DRG® Adiponectin (human) ELISA (EIA-4177)

Revised 11 Jan. 2011 rm (Vers. 5.1) **RUO**

This kit is intended for Research Use Only

This kit is not intended for in vitro diagnostic use.

INTENDED USE

This Human Adiponectin (ACRP30) ELISA kit is used for the non-radioactive determination of Human Adiponectin in serum, plasma, and adipocyte extracts or cell culture media samples. This kit specifically measures native Human Adiponectin and has no cross reactivity to Mouse Adiponectin. One kit is sufficient to measure 38 unknown samples in duplicate.

REAGENTS SUPPLIED

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

A. Human Adiponectin ELISA Plate
Coated with Mouse anti-Human 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: 1 sheet
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. Human Adiponectin Standard
Purified Recombinant Human Adiponectin, lyophilized.
Quantity: 100 ng
Preparation: Contents Lyophilized. Reconstitute with 0.5 mL distilled or deionized water to obtain 200 ng/mL.

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

F. 10X Assay Buffer (Sample Diluent)
100mM Phosphate buffer, pH 7.5, containing 0.08% Sodium Azide, 1% BSA Quantity: 50 mL Preparation: Dilute 1:10 with distilled or deionized water to make 1X Assay Buffer
Note: Use 1X Assay Buffer to dilute samples (Section SAMPLE PREPARATION) and Standard Curve (Section STANDARD AND QUALITY CONTROLS PREPARATION)

G. Assay Buffer A (Assay Running Buffer)
0.05M Phosphosaline containing 0.025M EDTA, 0.08% Sodium Azide, 1% BSA Quantity: 7 mL Preparation: Ready to Use

H. Human Adiponectin Detection Antibody
Pre-titered Biotinylated Mouse anti-Human Adiponectin Antibody
Quantity: 3 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

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.

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.

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 µL ~ 50 µL and 50 µL ~ 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

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 3,000xg 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.

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.

STANDARD AND QUALITY CONTROLS PREPARATION

A. Human Adiponectin Standard Preparation

1. Use care in opening the lyophilized Standard vial.
   Using a pipette, reconstitute the Human 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 seven tubes 100, 50, 25, 12.5, 6.25, 3.125, 1.56 ng/mL.
   Add 0.2 mL Assay Buffer (Sample Diluent) to each of the seven tubes.
   Prepare serial dilutions by adding 0.2 mL of the 200 ng/mL reconstituted standard to the 100 ng/mL tube, mix well and transfer 0.2 mL of the 100 ng/mL reconstituted standard to the 50 ng/mL tube, mix well and transfer 0.2 mL of the 50 ng/mL Standard to the 25 ng/mL tube, mix well and transfer 0.2 mL of the 25 ng/mL Standard to the 12.5 ng/mL tube, mix well and transfer 0.2 mL of the 12.5 ng/mL Standard to the 6.25 ng/mL tube, mix well and transfer 0.2 mL of the 6.25 ng/mL Standard to the 3.125 ng/mL tube, mix well and transfer 0.2 mL of the 3.125 ng/mL Standard to the 1.56 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 Assay Buffer (Samples Diluent) to Add	Volume of Standard to Add
100	0.2 mL	0.2 mL of 200 ng/mL
50	0.2 mL	0.2 mL of 100 ng/mL
25	0.2 mL	0.2 mL of 50 ng/mL
12.5	0.2 mL	0.2 mL of 25 ng/mL
6.25	0.2 mL	0.2 mL of 12.5 ng/mL
3.125	0.2 mL	0.2 mL of 6.25 ng/mL
1.56	0.2 mL	0.2 mL of 3.125 ng/mL

B. Human Adiponectin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials.
Using a pipette, reconstitute each of the Human 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.

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 content of each bottle of Wash Buffer with 450 mL deionized water (dilute both bottles 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 60 µL Assay Buffer A to all wells.
4. Add in duplicate 20 µL Assay Buffer A to blank wells.
5. Add in duplicate 20 µL Human 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.
6. Add 20 µL Detection Antibody to all wells. **For best result all additions should be completed within one hour.** 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.
7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
8. 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.
9. 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.
10. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
11. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
12. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5 to 20 minutes. Blue color should be formed in wells of Adiponectin standards with intensity proportional to increasing concentrations of 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.
13. 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 590nm 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 for Human Adiponectin ELISA

	Step 1	Step 2	Step 3-4	Step 5	Step 6	Step 6-8	Step 9	Step 10-11	Step 12	Step 12	Step 13	Step 13
Well #	Dilute each bottle of 10X Wash Buffer with 450mL Deionized Water.	Wash plate 3X with 300 µl Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels	Assay Buffer A	Standards/Controls/	Detection Ab	Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µl Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 5X with 300 µl Wash Buffer	Substrate	Seal, Agitate, Incubate 5 - 10 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			80 µl		20 µl		100 µl		100 µl		100 µl	
C1, D1			60 µl	20 µl of 1.56 ng/mL Standard								
E1, F1			60 µl	20 µl of 3.125 ng/mL Standard								
G1, H1			60 µl	20 µl of 6.25 ng/mL Standard								
A2, B2			60 µl	20 µl of 12.5 ng/mL Standard								
C2, D2			60 µl	20 µl of 25 ng/mL Standard								
E2, F2			60 µl	20 µl of 50 ng/mL Standard								
G2, H2			60 µl	20 µl of 100 ng/mL Standard								
A3, B3			60 µl	20 µl of QC I								
C3, D3			60 µl	20 µl of QC II								
E3, F3			60 µl	20 µl of Sample								
G3, H3			60 µl	20 µl of Sample								
A4, B4 ↓			60 µl	20 µl of Sample								

MICROTITER PLATE ARRANGEMENT

Human Adiponectin ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	12.5 ng/mL	QC 1									
B	Blank	12.5 ng/mL	QC 1									
C	1.56 ng/mL	25 ng/mL	QC 2									
D	1.56 ng/mL	25 ng/mL	QC 2									
E	3.125 ng/mL	50 ng/mL	Sample 1									
F	3.125 ng/mL	50 ng/mL	Sample 1									
G	6.25 ng/mL	100 ng/mL	Sample 2									
H	6.25 ng/mL	100 ng/mL	Sample 2									

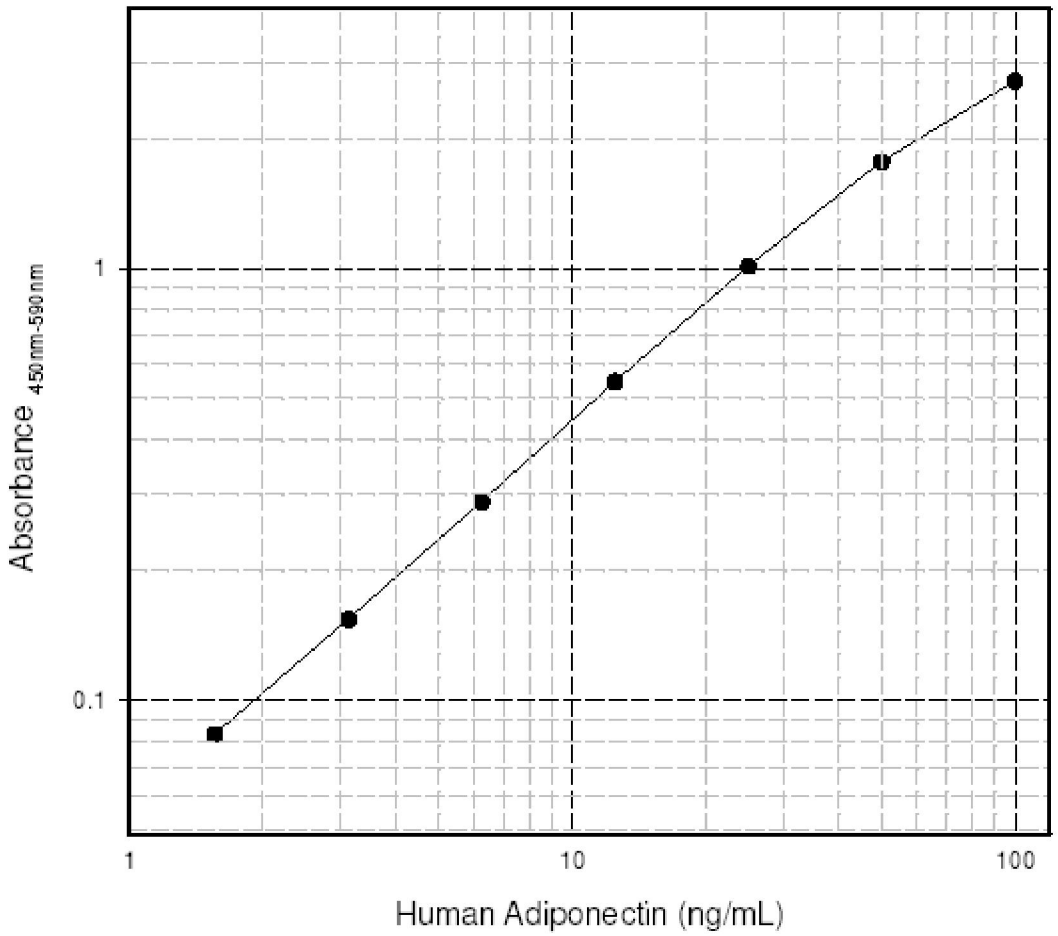
CALCULATIONS

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).

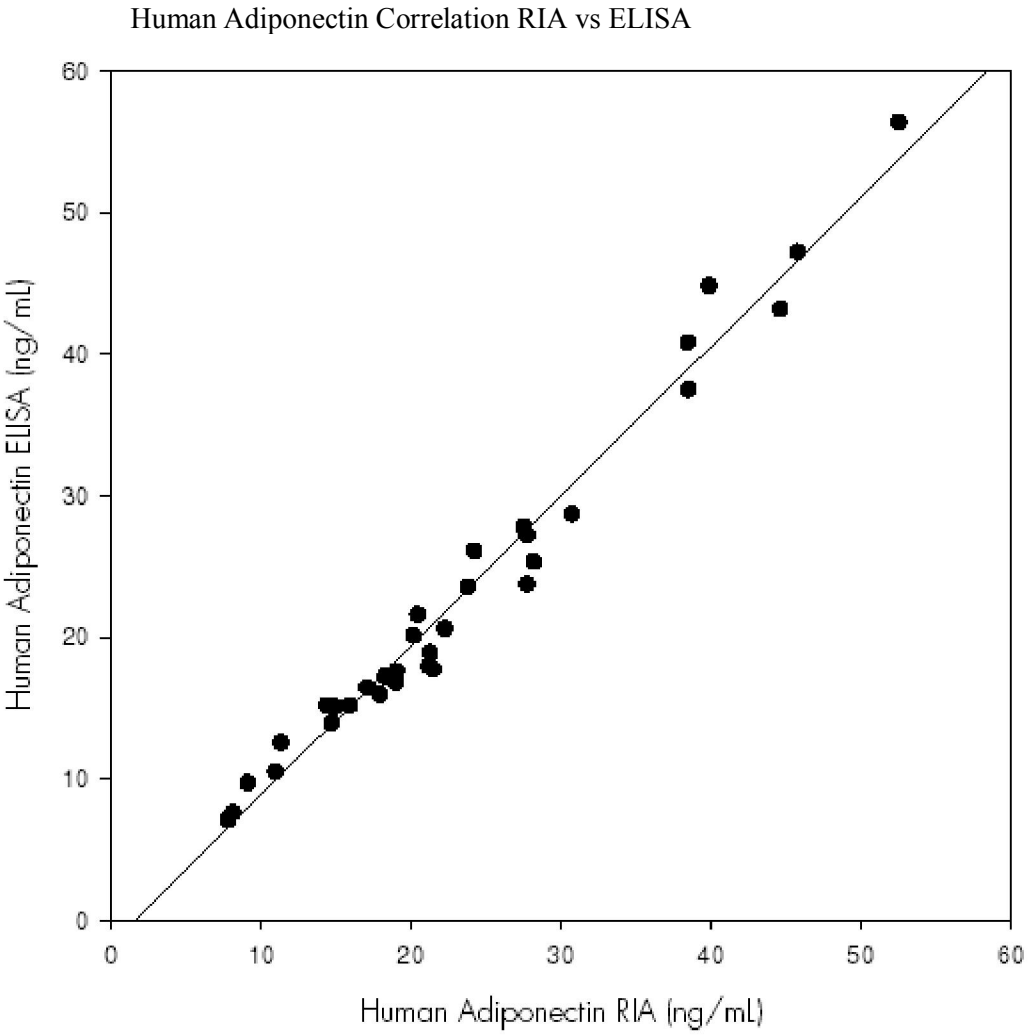
STANDARD CURVE

Human Adiponectin ELISA



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

CORRELATION GRAPH



Serum samples obtained from 33 human subjects were assayed for Adiponectin content using both DRG Human Adiponectin RIA Kit and DRG Human Adiponectin ELISA Kit. Correlation of the two kits is derived by linear regression analysis of paired results from each sample.

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.

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.

Material Safety Data Sheets (MSDS)

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

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