

Human Adiponectin ELISA Kit

Catalog No. CKH117 **1 x 96 tests**

Introduction: Human Adiponectin /Acrp30 is expressed and secreted exclusively by adipose tissue. Adiponectin is a hormone that plays a role in the regulation of glucose and lipid homeostasis. Adiponectin as a potent insulin enhancer can link adipose tissue and whole-body glucose metabolism. Levels of Adiponectin are decreased in obesity and it has been observed that Adiponectin levels are also reduced in patients with coronary artery disease.

The Cell Sciences® Human Adiponectin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Adiponectin in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human Adiponectin coated on a 96-well plate. Standards and samples are pipetted into the wells and Adiponectin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Adiponectin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Adiponectin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents:

1. Adiponectin Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human Adiponectin.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials of recombinant human Adiponectin.
4. Assay Diluent A (Item D): 2 bottles of 30 ml diluent buffer, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent C (Item L): 2 bottles of 15 ml of diluent buffer. For Standard/ Sample (cell culture medium/urine) diluent.
6. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Detection Antibody and HRP-Streptavidin Concentrate diluent.
7. Detection Antibody Adiponectin (Item F): 2 vial of biotinylated antihuman Adiponectin (each vial is enough to assay half microplate).
8. HRP-Streptavidin Concentrate (Item G): 8 µl 4000x concentrated HRP-conjugated streptavidin.
9. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
10. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

Storage: May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.



**Additional
Materials
Required:**

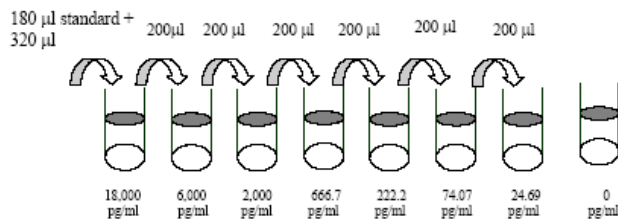
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 μ l to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

**Reagent
Preparation:**

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: Assay Diluent A (Item D) is used for dilution of serum/plasma samples, serum/plasma samples require around 30,000-fold dilution. Assay Diluent C can be used for dilution of cell culture supernates/urine.

For example, add 2 μ l of serum/plasma into a tube with 398.0 μ l Assay Diluent A to prepare a 200-fold diluted sample. Mix through and then pipette 2 μ l of prepared 200-fold diluted sample into a tube with 298 μ l 1x Assay Diluent A to prepare a final 30,000 fold diluted sample.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C and then add 400 μ l Assay Diluent A (for serum/plasma samples) or Assay Diluent C (for cell culture supernates/urine) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 180 μ l Adiponectin standard (50 ng/ml) from the vial of Item C, into a tube with 320 μ l Assay Diluent A or Assay Diluent C to prepare a 18,000 pg/ml stock standard solution. Pipette 400 μ l Assay Diluent A or Assay Diluent C into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent A or Assay Diluent C serves as the zero standard (0 pg/ml). The 18,000 pg/ml standard in Assay Diluent A may be saturated, we recommend to start from 6,000 pg/ml for Assay Diluent A Standard curve.



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 4,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 3 μ l of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 4,000 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use).

**Assay
Procedure:**

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ l of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 μ l) using a multi-channel Pipette 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.
4. Add 100 μ l of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 μ l of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 μ l of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 μ l of Stop Solution (Item I) to each well. Read at 450 nm immediately.



Assay Procedure Summary:

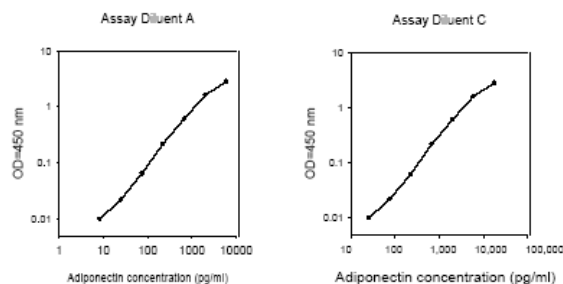
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 μ l standard or sample to each well.
Incubate 2.5 hours at room temperature or over night at 4°C.
3. Add 100 μ l prepared biotin antibody to each well.
Incubate 1 hour at room temperature.
4. Add 100 μ l prepared Streptavidin solution.
Incubate 45 minutes at room temperature.
5. Add 100 μ l TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.
6. Add 50 μ l Stop Solution to each well.
Read at 450 nm immediately.

Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of Adiponectin is typically less than 25 pg/ml.



C. RECOVERY

Recovery was determined by spiking various levels of human Adiponectin into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	93.57	83-104
Plasma	90.29	80-103
Cell culture media	96.59	83-107

D. LINERATY

Sample Type	Serum	Plasma	Cell Culture Media
1:2 Average % of Expected Range (%)	95 83-104	94 84-103	92 82-103
1:4 Average % of Expected Range (%)	93 84-104	92 83-102	93 85-104

REPRODUCIBILITY

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Specificity:

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- γ , Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 α , MIP-1 β , MIP-1 δ , PARC, PDGF, RANTES, SCF, TARC, TGF- β , TIMP-1, TIMP-2, TNF- α , TNF- β , TPO, VEGF).



Troubleshooting Guide:

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at <-20°C after reconstitution, others at 4 °C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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