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Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human Adiponectin (Acrp30)

Cat. No. 442307

ELISA Kit for Accurate Quantitation of Human Adiponectin from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

Adiponectin is also referred to as Acrp30, AdipoQ, apM1 and gelatin binding protein of 28 kDa (GBP-28), which in humans is encoded by the ADIPOQ gene. Adiponectin contains 244 amino acids with a collagen-like domain at the N-terminus and a globular domain at the C-terminus. The globular domain can be proteolytically truncated forming the globular adiponectin (gAdiponectin). This globular domain resembles the structure of the complement C1q and the TNF-alpha superfamily. Adiponectin exists in a wide range of homo-oligomers: a low-molecular weight (LMW) trimer, a medium molecular weight (MMW) hexamer and a high molecular weight (HMW) multimer with little or no interconversion between the forms. Females generally have higher HMW than males in both proportion and absolute amounts. Adiponectin has two receptors: adipoRs-1 and -2. AdipoR1 mRNA is detectable in skeletal muscle, spleen, lung, heart, kidney, and liver. AdipoR2 is expressed in liver, but also detectable in heart, lung, skeletal muscle, and kidney.

Adiponectin plays an important role in carbohydrate and lipid metabolism. It promotes insulin sensitivity and protects cells from inflammation and apoptosis. It is also hypothesized to have anti-atherogenic activity. Normal circulating adiponectin level ranges from 4-30 ug/mL. Adiponectin levels are inversely correlated with body mass index (BMI). Low serum levels of adiponectin are found in obesity, type 2 diabetes and coronary artery disease. Therefore, adiponectin serves as an important clinical biomarker for metabolic syndromes.

The LEGEND MAX™ Human Adiponectin (Acrp30) ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human adiponectin antibody. The Detection Antibody is a biotinylated mouse monoclonal anti-human adiponectin antibody. This kit is specifically designed for the accurate quantitation of human adiponectin (including globular, LMW, MMW and HMW adiponectin) from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

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Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Human Adiponectin Pre-coated 96 well Strip Micro-plate	1 plate		77533
Human Adiponectin Detection Antibody	1 bottle	12 mL	77534
Human Adiponectin Standard	1 vial	lyophilized	76830
Avidin-HRP B	1 bottle	12 mL	78230
Assay Buffer B	2 bottles	25 mL	79128
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP B	
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer. *Assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.*

Plasma: Collect blood samples in citrate, heparin or EDTA-containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. *Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles*.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and mix until dissolved.

2. Reconstitute the lyophilized Human Adiponectin Standard by adding the volume of Assay Buffer B to make the 100 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.

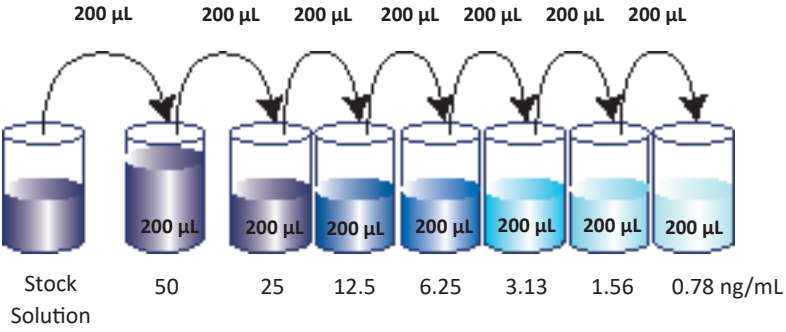
3. For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilutions are necessary, samples should be diluted in the corresponding cell culture medium.

4. It is recommended that serum or plasma samples be diluted by 1000-fold in Assay Buffer B (Additional Assay Buffer B is available on custom basis, part No. 79128). We recommend a two-step dilution: e.g. dilute 5 uL sample into 120 uL Assay Buffer B for the first 25-fold dilution and then further dilute 5 uL into 195 uL Assay Buffer B for another 40-fold dilution to achieve a final 1000-fold dilution.

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit. Do not shake the plate during incubations.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 400 µL of the 50 ng/mL top standard by diluting 200 µL of the standard stock solution in 200 µL of Assay Buffer B. Perform six two-fold serial dilutions of the 50 ng/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human adiponectin standard concentrations in the tubes are 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 1.56 ng/mL, and 0.78 ng/mL respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples.
5. Add 50 µL of standard dilutions or samples to the appropriate wells.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 1 hour without shaking.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer. Wash the plate with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
8. Add 100 µL of Human Adiponectin Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour without shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 7.

10. Add 100 µL of Avidin-HRP B solution to each well, seal the plate and incubate at room temperature for 30 minutes without shaking.

11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 7. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

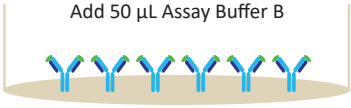
12. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human adiponectin should turn blue in color with intensity proportional to concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The well color should change from blue to yellow.

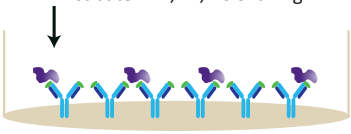
14. Read absorbance at 450 nm within 20 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

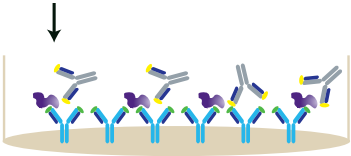
1. Add 50 µL Assay Buffer B



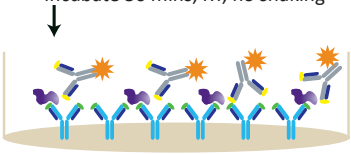
2. Add 50 µL diluted standards or samples
Incubate 1 hr, RT, no shaking



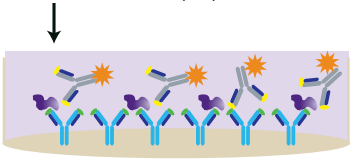
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, no shaking



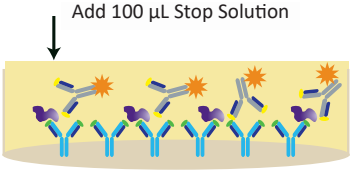
4. Wash 4 times
Add 100 µL Avidin-HRP B solution
Incubate 30 mins, RT, no shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 15 mins, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

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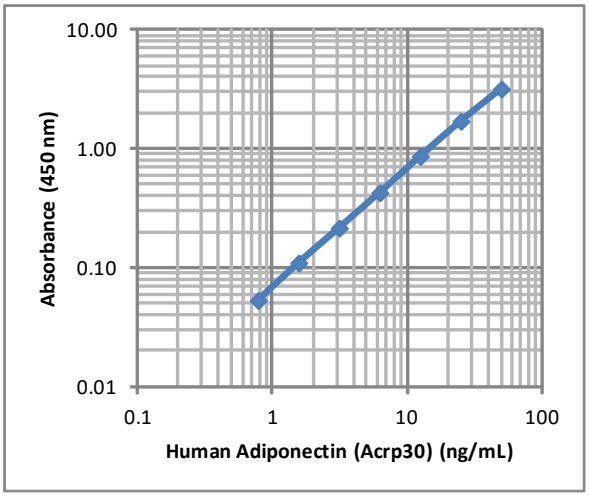
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the sample concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at 50 ng/mL:

Human	PDGF-BB, TNF-α, GM-CSF, IL-6, FASL, LT-α, TWEAK, TGF-β1, IL-17A, IL-17A/F, IL-22, IL-5, EPO, IL-4, MCP-4, BCA-1/BLC, I-309, MCP-1, Eotaxin, NAP-2, MIG, IP-10, ITAC, GRO-α, GRO-β, Groγ, CXCL5, Eotaxin-2, TECK, Eotaxin-3, CTACK, MEC, GCP-2, SDF-1α, RANTES, MCP-2, Beta-trophin, Cystatin C, and APRIL
Mouse	Adiponectin and TNF-α

No detectable signal was observed when tested with rat (Sprague Dawley, Long-Evans), equine, bovine and mouse (CD-1, C57/BL6) sera.

There was detectable signal in rhesus monkey serum (equivalent to 34.0 ug/mL), cynomolgus monkey serum (equivalent to 39.5 ug/mL) and porcine serum (equivalent to 1.1 ng/mL).

Sensitivity: The average minimum detectable concentration of human adiponectin is 0.086 ng/mL.

Recovery: 8 human serum and 8 plasma samples were diluted with Assay Buffer B to produce sample concentrations within the dynamic range of the assay. Three levels of recombinant Human adiponectin (12.5 ng/mL, 6.25 ng/mL and 1.56 ng/mL) were then spiked in, and analyzed with the LEGEND MAX™ Human Adiponectin ELISA Kit. On average, 97% of the adiponectin was recovered from serum and plasma samples.

Linearity: Ten human serum and plasma samples were diluted with Assay Buffer B to produce sample concentrations within the dynamic range of the assay. On average, 100% of the expected adiponectin was detected from serum samples and plasma samples.

Intra-Assay Precision: Diluted serum samples of high, median and low concentrations of human adiponectin were tested with 16 replicates in one assay.

	Sample 1	Sample 2	Sample 3
Number of Replicates	16	16	16
Mean Concentration (ng/mL)	20.0	5.2	1.3
Standard Deviation	1.0	0.2	0.1
% CV	4.9	4.3	4.5

Inter-Assay Precision: Diluted serum samples of high, median and low concentrations of human adiponectin were tested in four independent assays.

	Sample 1	Sample 2	Sample 3
Number of Replicates	4	4	4
Mean Concentration (ng/mL)	18.1	4.8	1.2
Standard Deviation	0.2	0.2	0.1
% CV	1.1	5.0	10.0

Biological Samples:

Serum and Plasma

Normal human serum (n=20) and plasma (n=20) samples were tested for endogenous adiponectin. The concentrations measured are shown below:

	Serum (n=20)	EDTA Plasma (n=10)	Heparin Plasma (n=5)	Citrate Plasma (n=5)
Detectable %	100	100	100	100
Mean (ug/mL)	25.8	16.3	14.5	14.9
Maximum (ug/mL)	44.3	27.1	24.3	19.3
Minimum (ug/mL)	7.3	3.8	8.8	8.7

Cell Culture Supernatant

Culture medium of human pre-adipocytes and adipocytes from a commercial source were measured for adiponectin concentrations. Human subcutaneous preadipocytes were obtained from adipose tissue. Culture medium was prepared from confluent preadipocytes or 2-3 week old differentiated adipocytes after 24hrs incubation. The culture supernatant was immediately flash frozen in liquid nitrogen and stored at -80°C.

There was no detectable amount of adiponectin in human pre-adipocyte culture medium, but 95.6 ng/mL adiponectin detected in the differentiated adipocyte.

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature or timing	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
	Plate was shaken during the assay	Rerun the assay without shaking the plate
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes

Notes

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LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
8999 BioLegend Way
San Diego, CA 92121
Tel: 1.858.768.5800
Tel US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com

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