Human Adiponectin ELISA Kit

Introduction

Adiponectin, also known as Adipocyte Complement-Related Protein of 30kDa (ACRP30), is a secreted serum protein expressed exclusively in differentiated adipocytes. Studies indicated that decreased plasma adiponectin concentration is associated with obesity, insulin resistance (1), essential hypertension (2), inflammation and atherosclerosis (3), and acute myocardial infarction (4). On the other hand, increased adiponectin level leads to nephrotic syndrome (5, 6).

Principal of the Assay

The Human Adiponectin ELISA Kit is designed for detection of adiponectin in human urine, plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures adiponectin in less than 4 hours. A polyclonal antibody specific for adiponectin has been pre-coated onto a microplate. Adiponectin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for adiponectin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Adiponectin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human adiponectin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Adiponectin Standard:** Human adiponectin in a buffered protein base (800 ng, lyophilized).
- **Biotinylated Adiponectin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against adiponectin (80 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 botlles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).

- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2 - 8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:500 into MIX Diluent. The undiluted samples can be stored at -200C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:500 into MIX Diluent. The undiluted samples can be stored at -200C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Dilute samples 1:10 into MIX Diluent. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x)**: Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8^{0}C.
- **Adiponectin Standard:** Reconstitute the 800 ng of human adiponectin Standard with 4 ml of MIX Diluent to generate a standard stock of 200 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by diluting stock (200 ng/ml) 1: 4 to produce a standard solution of 50 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the Standard solution (50 ng/ml) 1:2 with equal volume of MIX Diluent to produce 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Adiponectin] (ng/ml)
P1	1 part Stock (200 ng/ml) + 3 parts MIX Diluent	50.00
P2	1 part P1 + 1 part MIX Diluent	25.00
P3	1 part P2 + 1 part MIX Diluent	12.50
P4	1 part P3 + 1 part MIX Diluent	6.25
P5	1 part P4 + 1 part MIX Diluent	3.13
P6	1 part P5 + 1 part MIX Diluent	1.56
P7	1 part P6 + 1 part MIX Diluent	0.78
P8	MIX Diluent	0.00

- **Biotinylated Adiponectin Antibody (100x):** Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

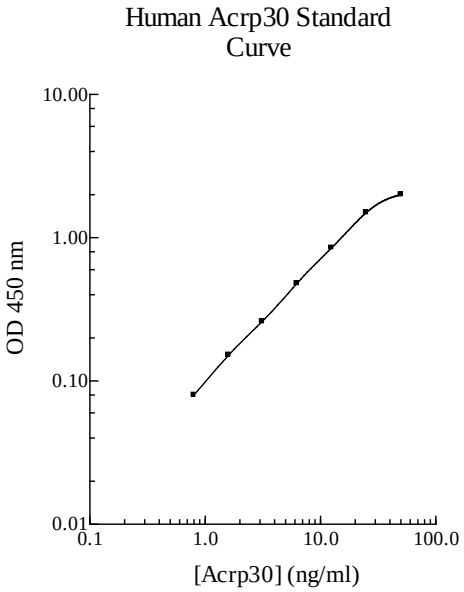
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated Adiponectin Antibody to each well and incubate for one hour.
- Wash a microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- This assay recognizes both natural and recombinant human adiponectin. It can detect both globular domain and full-length adiponectin.
- The minimum detectable dose of adiponectin is typically 0.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.2 % and 7.3 % respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:250	102%	97%
1:500	98%	101%
1:1000	105%	98%

	Average Percentage of Expected Value
Sample Dilution	**Cell Culture Media**
1:10	100%
1:20	98%
1:40	101%
1:80	106%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:5	106%
1:10	98%
1:20	93%

Recovery

Standard Added Value	1 – 25 ng/ml
Recovery %	95-113 %
Average Recovery %	101 %

Reference Value

- The normal blood levels of Adiponectin (ACRP30) range from 8.3-13.9 ug/ml.

References

(1) Tsao, T.S. *et al.* (2002) *EJP* 440:213-221
(2) Adamczak, M. *et al. AJH* 16:72-75
(3) Matsubara, M. *et al.* (2003) *Eur J Endocrinol.* 148(6): 657-62
(4) Kojima, S. *et al.* (2003) *Heart* 89(6): 667
(5) Zoccali, C. *et al.* (2003) *Kidney Int Suppl.* 84: S98-102
(6) Pannacciulli, N. *et al.* (2003) *J Clin Endocrinol Metab*. 88(4): 174