ADIPONECTIN ENZYME IMMUNOASSAY TEST KIT
Catalog Number: EA100013

ORIGENE

Enzyme Immunoassay for the Quantitative Determination of Adiponectin Concentration in Human Serum and Plasma

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

PRINCIPLE OF THE ASSAY

The Adiponectin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody pair directed against a distinct antigenic determinant on the adiponectin molecule. One mouse monoclonal anti-adiponectin antibody is used for solid phase immobilization (on the microtiter wells). Another mouse monoclonal anti-adiponectin antibody conjugated to horseradish peroxidase (HRP) is in the enzyme conjugate solution. The test samples are allowed to react sequentially with the two antibodies, resulting in the adiponectin molecules to be sandwiched between the solid phase and enzyme-linked antibodies. After two separate 60- minute incubation steps at room temperature, the wells are rinsed with Wash Buffer to remove unbound labeled antibodies. TMB Reagent is added and incubated for 30 minutes under dark conditions, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, changing the color to yellow. The concentration of adiponectin is directly proportional to the color intensity of the test samples. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-adiponectin
2. *250 ng/ml Adiponectin Standard (1.4 ml /vial)*
   250 ng/ml adiponectin in bovine serum with preservatives
3. *Standard Diluent (10 ml/vial, 1 vial)*
   For Standard Dilution Use ONLY
   Bovine Serum with preservatives
4. *Sample Diluent (50 ml/bottle, 1 bottle)*
   For Sample Dilution Use ONLY
   Contains phosphate buffer-BSA solution with preservatives
5. *Enzyme Conjugate Concentrate 100X (0.2ml/vial, 1 vial)*
   Contains mouse monoclonal anti-adiponectin conjugated to horseradish peroxidase
6. *Enzyme Conjugate Diluent (13 ml/vial)*
   For Enzyme Conjugate Concentrate Dilution Only
   Tris Buffer with Preservatives
7. *20X Wash Buffer (50 ml/bottle, 1 bottle)*
   Phosphate buffer with detergents
8. *TMB Reagent (11 ml/bottle, 1 bottle)*
   Contains one-step TMB solution
9. *Stop Solution (11 ml/bottle, 1 bottle)*
   Contains diluted hydrochloric acid (1N HCl)

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. For each test run, prepare a fresh standard set.
3. Please Note:
   a. Adiponectin Standards should be diluted with Standard Diluent.
   b. Samples should be diluted with Sample Diluent.
4. Prepare two-fold serial dilutions of the 250 ng/ml Standard with Standard Diluent:
   a. 250 ng/ml: Ready to use
   b. 125 ng/ml: 0.5 ml of 250 ng/ml + 0.5 ml of Standard Diluent
   c. 62.5 ng/ml: 0.5 ml of 125 ng/ml + 0.5 ml of Standard Diluent
   d. 31.25 ng/ml: 0.5 ml of 62.5 ng/ml + 0.5 ml of Standard Diluent
   e. 15.6 ng/ml: 0.5 ml of 31.25 ng/ml + 0.5 ml of Standard Diluent
   f. 7.8 ng/ml: 0.5 ml of 15.6 ng/ml + 0.5 ml of Standard Diluent
   h. 3.9 ng/ml: 0.5 ml of 7.8 ng/ml + 0.5 ml of Standard Diluent
   i. 0 ng/ml: 0.5 ml of Standard Diluent
5. *Samples need to be diluted 200-fold prior to use. Prepare a series of small tubes (i.e., 1.5 ml microcentrifuge tubes) and mix 5 µl of serum with 995 µl (0.995 ml) Sample Diluent.*
6. Working Conjugate Reagent: To prepare Working Adiponectin Conjugate Reagent, dilute the Enzyme Conjugate Concentrate (100X) with Enzyme Conjugate Diluent.

   Example: For 4 strips, prepare 4 ml of Working Enzyme Conjugate Reagent. Add 0.04 ml of Conjugate Concentrate (100X) to 3.96 ml of Conjugate Diluent.

   Do not reuse the Working Enzyme Conjugate Reagent. Make a fresh dilution before each assay.

7. Working Wash Buffer: Preparation of 1X Wash Buffer from 20X Stock. Add 50 ml of 20X Wash Buffer Stock to 950 ml of DI H_2O. The Working Wash Buffer is stable at 2-8°C for 30 days. NOTE: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

ASSAY PROCEDURE

1. *Prepare Standards. See Reagent Preparation.*
2. *Dilute samples 1:200 dilution. See Reagent Preparation.*
3. Secure the desired number of coated wells in the holder.
4. Dispense 100 µl of Adiponectin standards, and DILUTED specimens into the appropriate wells.
5. Incubate for 60 minutes at room temperature (18-25 °C).
6. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300 µl Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 100 µl of Adiponectin Working Enzyme Conjugate Reagent into each well.
8. Incubate for 60 minutes at room temperature (18-25 °C).
9. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300 ul Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.
10. Dispense 100 µl TMB solution into each well.
11. Incubate for 30 minutes at room temperature (18-25 °C).
12. Stop the reaction by adding 100 µl of Stop Solution into each well.
13. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
14. Read absorbance at 450 nm with a microtiter well reader *within 15 minutes*.

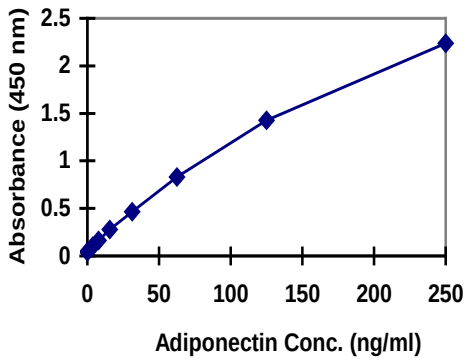
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. The corresponding concentration of Adiponectin (ng/ml) can be determined from the standard curve using the mean absorbance value for each sample. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The obtained values of samples should be multiplied by the dilution factor of 200 to obtain Adiponectin results in ng/ml.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450 nm shown on the Y axis against Adiponectin concentrations shown on the X axis. NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must generate its own data and standard curve in each experiment.

Adiponectin (ng/ml)	Absorbance (450 nm)
0	0.043
3.9	0.103
7.8	0.163
15.6	0.278
31.25	0.465
62.5	0.832
125	1.428
250	2.238



PERFORMANCE CHARACTERISTICS

1. Sensitivity

   The minimum detectable concentration of the Adiponectin ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.975 ng/ml.

2. Precision

   a. Intra-Assay Precision

   Within-run precision was determined by replicate determinations of three different plasma samples in one assay. Within-assay variability is shown below:

Plasma Sample	1	2	3
# Replicates	24	24	24
Mean Adiponectin (ug/ml)	3.0	5.6	10.2
S.D.	0.1	0.2	0.3
C.V. (%)	1.8%	3.4%	3.2%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different plasma samples over a series of individually calibrated assays. Between-assay variability is shown below:

Plasma Sample	1	2	3
# Replicates	40	40	40
Mean Adiponectin (ug/ml)	3.3	6.3	11.2
S.D.	0.4	0.4	0.9
C.V. (%)	11.2%	6.5%	8.0%

3. Recovery and Linearity Studies

a. Recovery

Various plasma samples of known Adiponectin levels were combined and assayed in duplicate. The mean recovery was 92.8%.

Pair Number	Expected Adiponectin (ug/ml)	Observed Adiponectin (ug/ml)	% Recovery
1	4.8	4.4	93.3%
2	7.3	6.7	91.6%
3	8.6	8.1	93.6%

b. Linearity

Three samples were serially diluted to determine linearity. The mean recovery was 99.2%.

#	Dilution	Expected Conc. (ug/ml)	Observed Conc. (ug/ml)	% Expected
1.	1:100	3.2	3.1	96.6%
	1:200		3.2	N/A
	1:400		3.5	108.8%
	1:800		3.4	105.5%
	1:1,600		3.5	108.1%
				Mean = 104.7%
2.	1:100	6.0	5.6	93.3%
	1:200		6.0	N/A
	1:400		6.1	101.5%
	1:800		6.1	100.9%
	1:1,600		5.9	98.0%
				Mean = 98.4%
3.	1:100	10.0	9.5	97.4%
	1:200		10.0	N/A
	1:400		9.7	97.0%
	1:800		9.4	93.7%
	1:1,600		9.2	92.2%
				Mean = 94.4%

TECHNICAL CONSULTATION

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