DIAGNOSTICS BIOCHEM CANADA

ADIPONECTIN ELISA

REF CAN-APN-5000

- *fast*
- *highly sensitive*
- *ready-to-use reagents*

DBC
Diagnostics Biochem Canada

WHY MEASURE ADIPONECTIN LEVELS?

Because Adiponectin has many functions...

In obesity and type II diabetes: Obesity is characterised by low serum adiponectin levels, while higher levels of adiponectin in plasma reduce the risk of type II diabetes (1). These results are important to predict the susceptibility to metabolic syndrome and insulin resistance (2).

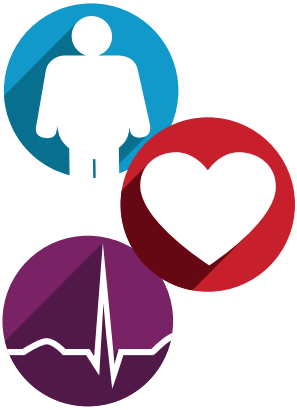
In cancer: Many cases of cancer are related to hormone levels. Adiponectin inhibits the progression of some cancers through its receptor (Adipo R1, Adipo R2) (3).

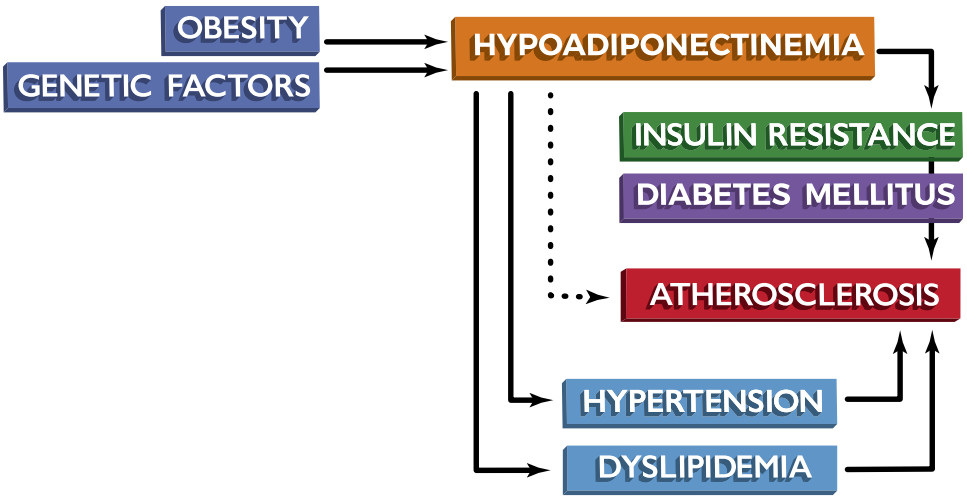
In cardiovascular diseases: It has been shown that patients with low levels of Adiponectin have an increased risk of developing coronary artery dieseases, as Adiponectin decreases the level of inflammation and chances of developing artherosclerosis (4).

In non-alcoholic fatty liver disease (NAFLD): NAFLD is characterized by insulin resistance and is associated with obesity, type II diabetes, and low levels of adiponectin (5, 6).

Adiponectin is a biomarker for:

Metabolic syndrome
Energy metabolism
Body weight regulation
Coronary artery disease and Atherosclerosis





ASSAY PRINCIPLE

The principle of the adiponectin ELISA is a two-step sandwich enzyme immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for adiponectin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of adiponectin is conjugated to biotin. During the first step, adiponectin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Unbound biotinylated antibody is removed by a washing. In the second step, streptavidin-HRP is added, which binds specifically to bound biotinylated antibody. Unbound streptavidin-HRP is removed by washing. Next, the enzyme substrate (TMB) is added. The colour intensity of the enzymatic reaction is directly proportional to the concentration of adiponectin. The enzymatic reaction is terminated by the addition of stopping solution.

The absorbance is measured on a microplate reader at 450 nm. The concentration of adiponectin in samples and controls can be calculated from of a plot of the standard curve, either graphically or by using immunoassay software.

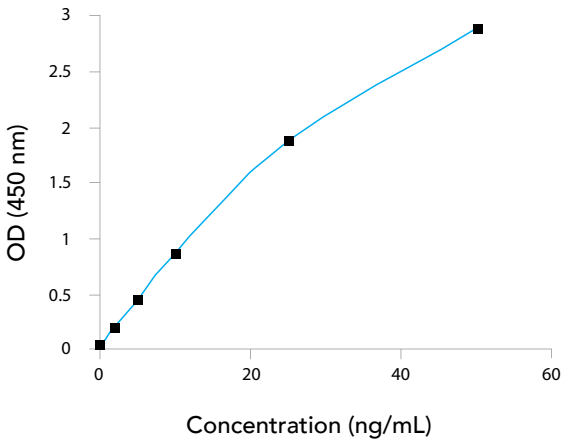
ASSAY PROCEDURE

- Dilute samples
- Add 50 µL calibrators/samples
- Add 100 µL of biotinylated antibody
- Incubate 1h at room temperature, with shaking
- Wash 3 times
- Add 100 µL of Streptavidin-HRP conjugate
- Incubate 30 mins. at room temperature, with shaking
- Wash 3 times
- Add 150 µL of TMB substrate
- Incubate 15 mins. at room temperature, with shaking
- Add 50 µL of stopping solution
- Read in a microplate reader at 450 nm

PERFORMANCE

Parameter	DBC	Competitor 1	Competitor 2
Total assay time	1h 45min	2h 30min	1h 45min
Ready to use reagents	Yes	No	No
Sample size, µL	50	100	100
Sample type	Serum, plasma	Serum, plasma	Serum, plasma
Precision, CV%			
Intra-assay	5.5–7.5	3.3–4.4	2.35–4.66
Inter-assay	6.6–8.4	5.8–6.2	5.8–6.72
Sensitivity			
LoD, ng/mL	0.055	0.47	0.6
LoQ, ng/mL	0.15	No data available	No data available

Typical Calibration Curve



PERFORMANCE

SPECIFICITY (CROSS-REACTIVITY)

The evaluation of the cross-reactivity was performed using proteins indicated in the table below. The results show that there is no significant cross-reactivity of the chosen analytes compared to adiponectin.

Proteins	Concentration (ng/mL)	(%) Cross-Reactivity
Leptin	100	0
TNF-α	100	0
IL-6	100	0.9
Resistin	100	0.1
C-peptide	10	0

INTERFERENCES

The following substances were tested and did not show significant interference in the Adiponectin assay: hemoglobin up to 0.25 g/L, bilirubin conjugated and free up to 85 µM, triglycerides up to 5.5 mg/mL and human serum albumin up to 60 g/L.

INTRA-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve.

Sample	Mean (µg/mL)	SD (µg/mL)	CV %
1	6.59	0.36	5.5
2	11.92	0.55	4.6
3	36.82	2.75	7.5

INTER-ASSAY PRECISION

Three samples were assayed 20 times each during 20 days.

Sample	Mean (µg/mL)	SD (µg/mL)	CV %
1	6.16	0.52	8.44
2	12.07	0.81	6.7
3	38.39	2.55	6.6

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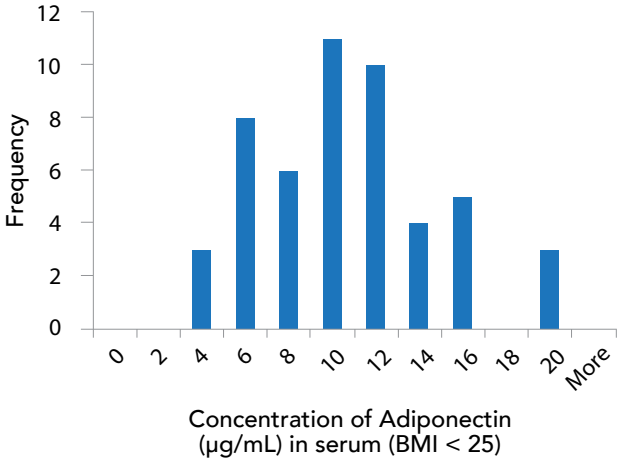

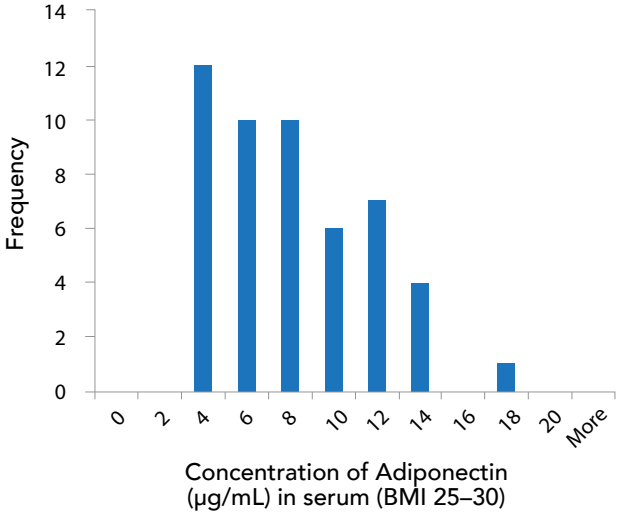

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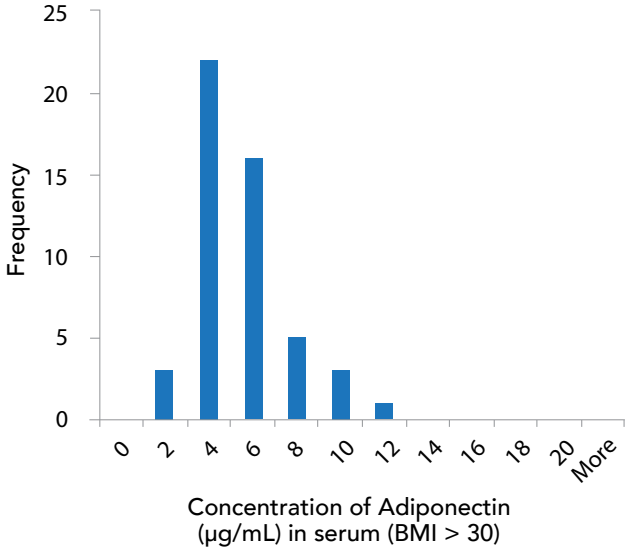
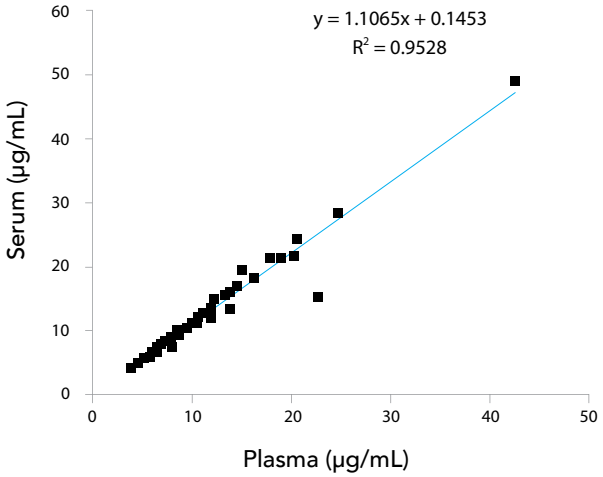


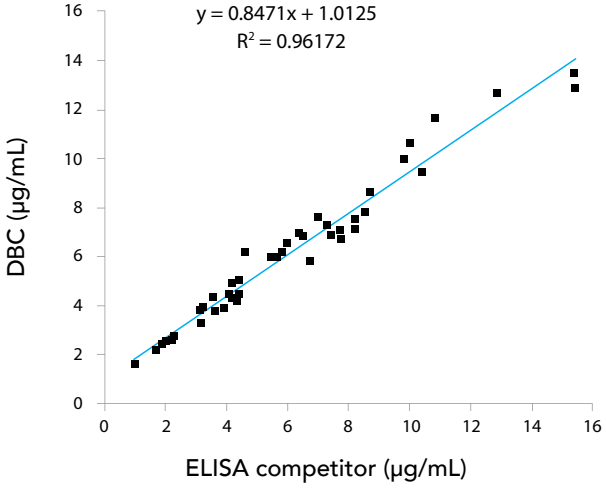
Group	N	Mean (µg/mL)	95% Confidence range (µg/mL)
BMI < 25	50	9.7	3.4–19.5
BMI 25–30	50	7.1	2.6–13.7
BMI > 30	50	4.5	1.8–9.4

SAMPLE MATRIX COMPARISON



DBC Adiponectin ELISA kit can be used for serum and plasma samples.

COMPARATIVE STUDIES



The new device was compared to a leading ELISA kit in the market. As shown, the comparison was performed with 40 human serum samples. The results show that the correlation coefficient (r) between the two methods is 0.98.

LITERATURE

1. Haluzik M, Parizková J, Haluzic MM. Adiponectin and its role in the obesity-induced insulin resistance and related complications. *Physiol Res*. 2004; 53(2):123–9.
2. Blümer RM, Van der Crabben SN, Stegenga ME, et al. Hyperglycemia prevents the suppressive effect of hyperinsulinemia on plasma adiponectin levels in healthy humans. *Am J Physiol Endocrinol Metab*. 2008; 295(3):E613–7.
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4. Lu G, Ghiem A, Anuurad E, et al. Adiponectin levels are associated with coronary artery diseases across Caucasian and African-American ethnicity. *Transl Res*. 2007; 149(6):317–23.
5. Shimada M, Kawahara H, Ozaki K, et al. Usefulness of a combined evaluation of serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of non-alcoholic steatohepatitis. *Am J Gastroenterol*. 2007; 102(9):1931–8.
6. Younossi ZM, Jarrar M, Nugent C, et al. A novel diagnostic biomarker panel for obesity-related non-alcoholic steatohepatitis (NASH). *Obest Surg*. 2008; 18(11):1430–7.

DBC ADIPONECTIN at a glance

REF CAN-APN-5000

Sample Volume: 50 µL

Total Assay Time: 105 minutes

Sensitivity: 0.055 ng/mL

FOR MORE INFORMATION, PLEASE CONTACT DBC AT:

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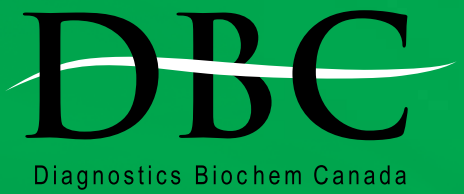

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