Diagnósticos Biochem Canadá

Adiponectin

ELISA

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

REF CAN-APN-5000
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 diseases, 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
- Atherosclerosis

**LITERATURE**

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

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

### Typical Calibration Curve

![Typical Calibration Curve](image-url)

**Graph Details:**
- **X-axis:** Concentration (ng/mL)
- **Y-axis:** OD (450 nm)
- **Data Points:** Concentration of Adiponectin (µg/mL) in serum (BMI > 30)

**Reference Range:**

**BMI < 25**: 9.7 ± 3.4–19.5

**BMI 25–30**: 7.1 ± 2.6–13.7

**BMI > 30**: 4.5 ± 1.8–9.4
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.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Concentration (ng/mL)</th>
<th>(%) Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>IL-6</td>
<td>100</td>
<td>0.9</td>
</tr>
<tr>
<td>Resistin</td>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>C-peptide</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (µg/mL)</th>
<th>SD (µg/mL)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.59</td>
<td>0.36</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>11.92</td>
<td>0.55</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>36.82</td>
<td>2.75</td>
<td>7.5</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION

Three samples were assayed 20 times each during 20 days.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (µg/mL)</th>
<th>SD (µg/mL)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.16</td>
<td>0.52</td>
<td>8.44</td>
</tr>
<tr>
<td>2</td>
<td>12.07</td>
<td>0.81</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>38.39</td>
<td>2.55</td>
<td>6.6</td>
</tr>
</tbody>
</table>
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### Reference Range

**Concentration of Adiponectin (µg/mL) in serum (BMI > 30)**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean (µg/mL)</th>
<th>95% Confidence range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt; 25</td>
<td>50</td>
<td>9.7</td>
<td>3.4–19.5</td>
</tr>
<tr>
<td>BMI 25–30</td>
<td>50</td>
<td>7.1</td>
<td>2.6–13.7</td>
</tr>
<tr>
<td>BMI &gt; 30</td>
<td>50</td>
<td>4.5</td>
<td>1.8–9.4</td>
</tr>
</tbody>
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**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.


ADIPONECTIN

WHY MEASURE

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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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