Adiponectin is an adipocyte-derived protein with diverse biological functions. It circulates in plasma as low molecular weight (LMW) trimers, medium molecular weight (MMW) hexamers, and high molecular weight (HMW) multimers. The different oligomeric forms have been suggested to exert distinct actions on specific target tissues. The HMW Adiponectin is the primary bioactive form with insulin-sensitizing activity and its circulating levels negatively correlate with obesity, insulin resistance and coronary artery disease (CAD). Current ELISA assays to detect HMW Adiponectin require the laborious process of selective enzyme digestion of the LMW and MMW forms. We have developed a novel ELISA that utilizes HMW Adiponectin-specific antibodies. This easy-to-use assay does not require any pretreatment of the sample and provides excellent reproducibility.

**GENERAL METHODS**

**• Antibody Generation**

HMW Adiponectin was purified from a heterogeneous mixture of recombinant Adiponectins using gel filtration chromatography and used to generate anti-HMW Adiponectin monoclonal antibodies. The antibodies were rigorously tested to ensure specificity for the HMW isorm of Adiponectin. Two antibodies specific for HMW Adiponectin were paired as the capture antibody and detection antibody in a standard quantitative sandwich ELISA. For the Measurement of High Molecular Weight Adiponectin (Catalog # DHWAD0) is a complete, fully validated, ready-to-run sandwich ELISA that provides the highest levels of specificity, accuracy, precision, and sensitivity in quantifying HMW Adiponectin.

**• Assay Procedure**

**A. ASAY PROCEDURE**

**• Detection of Adiponectin Isoforms in Human Serum using the Quantikine Human Total Adiponectin/Acrp30 and Quantikine Human HMW Adiponectin/Acrp30 Assay Kits.** The Human Total Adiponectin/Acrp30 Immunoassay detected all three molecular weight Adiponectin isoforms, while the Human HMW Adiponectin/Acrp30 Immunoassay only detected HMW Adiponectin. In addition, a clearer separation between the levels of Adiponectin in the different molecular weight classes was observed using the Human HMW Adiponectin/Acrp30 Immunoassay. The HMW Adiponectin fraction contained a small amount of HMW Adiponectin isoforms which were separated from the LMW and MMW isoforms. The HMW, MMW, and LMW forms of Adiponectin were purified from a heterogeneous mixture of recombinant Adiponectins using size exclusion chromatography. The HMW (green line), MMW (pink line), and LMW (red line) fractions, as well as a pool of molecular weight Adiponectin isoforms, were reapplied to a TSK gel chromatography column, and the individual fractions collected were analyzed using the Quantikine Human Total Adiponectin/Acrp30 and Quantikine Human HMW Adiponectin/Acrp30 Assay Kits.

**B. TABLE 1**

**Intra-Assay Precision**

<table>
<thead>
<tr>
<th>Sample</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>21.4</td>
<td>23.8</td>
<td>20.7</td>
<td>23.7</td>
</tr>
<tr>
<td>CoV (%)</td>
<td>7.4</td>
<td>8.4</td>
<td>6.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**C. FIGURE 2**

**Detects of Adiponectin and Isoforms in Human Serum using the Quantikine® Human Total Adiponectin/Acrp30 and Quantikine® Human HMW Adiponectin/Acrp30 ELISA Kits.** The Human HMW Adiponectin/Acrp30 Immunoassay detected HMW Adiponectin only, while the Human Total Adiponectin/Acrp30 Immunoassay detected all three molecular weight Adiponectin isoforms. The levels of Adiponectin in serum from individuals with low BMI, high BMI, and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 ELISA Kit. A 2.4 fold and a 5.1 fold difference in Adiponectin levels between low BMI and very high BMI (green bar) were calculated for each ELISA kit. The Human Total Adiponectin/Acrp30 immunoassay detected a 1.9 fold and a 2.9 fold difference in Adiponectin levels between individuals with low and high BMI, and low and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 ELISA Kit.

**D. FIGURE 3**

**Detects of Adiponectin and Isoforms in Human Serum using the Quantikine® Human Total Adiponectin/Acrp30 and Quantikine® Human HMW Adiponectin/Acrp30 ELISA Kits.** The Human HMW Adiponectin/Acrp30 Immunoassay detected HMW Adiponectin only, while the Human Total Adiponectin/Acrp30 Immunoassay detected all three molecular weight Adiponectin isoforms. The levels of Adiponectin in serum from individuals with low BMI, high BMI, and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 ELISA Kit. A 2.4 fold and a 5.1 fold difference in Adiponectin levels between low BMI and very high BMI (green bar) were calculated for each ELISA kit. The Human Total Adiponectin/Acrp30 immunoassay detected a 1.9 fold and a 2.9 fold difference in Adiponectin levels between individuals with low and high BMI, and low and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 ELISA Kit. A 2.4 fold and a 1.9 fold difference in Adiponectin levels between individuals with high and low BMI, and low and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 ELISA Kit.

**E. FIGURE 4**

**Assay Linearity.** Serum (100 µL) was incubated using EDTA (green line) or heparin (gold line) as an anticoagulant. Serum samples were analyzed using the Quantikine Human HMW Adiponectin/Acrp30 ELISA Kit. The percent of obtainable recovery was calculated for all samples (n=30).