Human Adiponectin ELISA Kit

Enzyme Immunoassay for the quantification of Human Adiponectin in serum, plasma, cell culture supernatants

Catalog number: ARG80131
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INTRODUCTION

This gene is expressed in adipose tissue exclusively. It encodes a protein with similarity to collagens X and VIII and complement factor C1q. The encoded protein circulates in the plasma and is involved with metabolic and hormonal processes. Mutations in this gene are associated with adiponectin deficiency. Multiple alternatively spliced variants, encoding the same protein, have been identified. [provided by RefSeq]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Adiponectin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Adiponectin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Adiponectin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Adiponectin in the sample is then determined by comparing the O.D of samples to the standard curve.
MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microplate</td>
<td>8 X 12 strips</td>
<td>4°C. Unused strips should be sealed tightly in the air-tight pouch.</td>
</tr>
<tr>
<td>Standard (Lyophilized)</td>
<td>3 X 10 ng/vial</td>
<td>4°C</td>
</tr>
<tr>
<td>Standard diluent buffer</td>
<td>20 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>Antibody conjugate concentrate</td>
<td>1 vial (400 µl)</td>
<td>4°C</td>
</tr>
<tr>
<td>Antibody diluent buffer</td>
<td>16 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>HRP-Streptavidin concentrate</td>
<td>1 vial (400 µl)</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>HRP-Streptavidin diluent buffer</td>
<td>16 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>20X Wash buffer</td>
<td>50 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB substrate</td>
<td>12 ml</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>STOP solution</td>
<td>12 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>Plate sealer</td>
<td>6 strips</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately.

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or aliquot and store samples at \( \leq -20 \) °C. Avoid repeated freeze-thaw cycles.

**REAGENT PREPARATION**

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X Antibody conjugate**: Dilute 30X antibody conjugate concentrate into 1X antibody diluent buffer to yield 1X Detection antibody solution.
- **1X HRP-Streptavidin Solution**: Dilute 30X HRP-Streptavidin concentrate solution into 1X HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- **Standards**: Reconstitute the standard with 1 ml standard diluent buffer to yield a stock concentration of 10000 pg/ml. Make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.

![Dilution diagram](image_url)
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 μl of standards, samples and zero controls (standard diluent buffer) into wells. Incubate for 1.5 h at 36 °C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (350 μl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 μl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 36°C.
5. Aspirate each well and wash as step 3.
6. Add 100 μl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate for 30 minutes at 36°C.
7. Aspirate each well and wash as step 3.
8. Add 100 μl of TMB Reagent to each well. Incubate for 15 minutes at 36°C in dark.
9. Add 100 μl of Stop Solution to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450nm immediately.
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.
QUALITY ASSURANCE

Sensitivity
The minimum detectable dose (MDD) of Human Adiponectin ranged from 62.5-4000 pg/ml. The mean MDD was 30 pg/ml.

Specificity
This assay recognizes natural and recombinant Human Adiponectin. No significant cross-reactivity or interference with the factors below was observed:
Mouse adiponectin

Intra-assay and Inter-assay precision
The CV values of both intra and inter precision fall below 10%.
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.