3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Adiponectin ELISA

Catalog Number **SE120138**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Adiponectin is an adipocyte-secreted hormone, consisting of 244 amino acids with a molecular mass of 28–30 kDa. It is one of the most abundant proteins in human blood, with circulating concentrations of 0.5–30 μL/mL, which accounts for ~0.01% of total plasma protein.[1] Adiponectin concentration is reversely associated with type 2 diabetes, coronary artery disease, and obesity, all together called the metabolic syndrome. Adiponectin decreases blood glucose and free fatty acid serum concentrations, and increases insulin sensitivity.[2] Adiponectin has been shown to have anti-inflammatory effects.[1] However, recent studies indicate that adiponectin may not be present in circulation as monomers or isolated globular forms, but rather in multimeric structures. The studies have shown the dominant forms of adiponectin that circulates in human blood are hexamers (LMW) and lager oligomers (HMW).[3,12-14] The LMW adiponectin level does not seem to differ between insulin sensitive and insulin resistant subjects, nor does LMW adiponectin differ between men and women. The increased levels of total adiponectin in insulin sensitive subjects and women were caused by increased amounts of HMW Adiponectin. Both total and HMW Adiponectin showed significant differences between the insulin sensitive and insulin resistant subjects.

The Adiponectin ELISA kit is used for the quantitative measurement of adiponectin in human serum or plasma. The Adiponectin ELISA is a solid phase sandwich enzyme immunoassay. Two monoclonal antibodies are directed against separate antigenic determinants on the adiponectin molecule. During incubation, adiponectin in the sample reacts with anti-Adiponectin antibodies bound to microwell plate wells and anti-Adiponectin antibodies bound to HRP. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3′,5,5′-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is red spectrophotometrically.

Components

Materials provided	96 Tests
Microwells coated with Anti-Adiponectin Ab	12 x 8 x 1
Adiponectin Calibrators Set (6 Vials)	0.25 mL
Enzyme Conjugate	12 mL
Wash Buffer	25 mL
TMB Substrate	12 mL
Stop Solution	12 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. Multiwell plate reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.
   Note: Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

Wash Buffer
Dilute contents of wash solution to 1,000 mL with distilled or deionized water in a suitable storage container. Store at room temperature (18–26 °C).

Working Substrate Solution
Pour the contents of the vial labeled Solution 'A' into the vial labeled 'B'. Mix and labeled accordingly.

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18–26 °C).

1. Format the microplate wells for each serum reference, control, and specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal, and store at 2–8 °C.
2. Pipette 25 µL of the appropriate serum reference, control, or specimen into the assigned well.
3. Add 100 µL of the enzyme conjugate reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Incubate 60 minutes at room temperature on a plate shaker set to 600 rpm.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 300 µL of wash buffer (see Preparation Instructions), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
7. Add 100 µL of working TMB substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells. Don't shake the plate after substrate addition.
8. Incubate at room temperature for fifteen (15) minutes.
9. Add 50 µL of Stop Solution to each well and mix gently for 30 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
10. Read the absorbance in each well at 450 nm (using a reference wavelength of 620–630 nm to minimize well imperfections) in a microplate reader. The results should be read within fifteen (15) minutes of adding the stop solution.

Results

Calculations

The standard curve is constructed as follows:

1. Check Adiponectin standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the Adiponectin standards (vertical axis) versus the Adiponectin standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	OD 450 nm	Concentration (µIU/mL)
Standard 1	.025	0
Standard 2	.096	3
Standard 3	.223	6
Standard 4	.497	15
Standard 5	1.194	35
Standard 6	2.095	70

Notes: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

References

1. Meier, U., and Gressner, A.M., (2004) Endocrine Regulation of Energy metabolism: Review of Pathobiochemical and Clinical Aspects of Leptin, Ghrelin, Adiponectin, and Resistin. Clin. Chem., 50: 1511-1525
2. Duntas, L.H. et al., (2004) Adiponectin: Novelties in Metabolism and Hormonal Regulation. Nutr. Neurosci., 7:195-200
3. Lara Castro, C. et al., (2006) Adiponectin multimeric Complexes and the Metabolic Syndrome Trait Cluster. Diabetes, 55:249-259

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