be measured in subjects at risk for type 2 diabetes, atherosclerosis and the metabolic syndrome.

3. PRECAUTIONS

Avoid contact with the acidic Stop Solution and the Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. This kit may contain components of human or animal origin. These materials should be handled as potentially infectious. Dispose consumable materials and unused components in accordance with national regulation requirements. Refer to the SDS for details (www.biovendor.com).

4. TECHNICAL HINTS

- Basic knowledge of ELISA principle is required
- Reagents with different lot numbers should not be mixed
- Use deionized (distilled) water, stored in clean containers
- Use only a quality Bovine Serum Albumin, Fraction V, Protease free
- It is necessary to use suitable diluents with respect to a particular type of samples. Validate diluents for the given type of sample prior to the batch measurement
- It is recommended to use a starting dilution of reagents (describe bellow). Lower and higher concentrations may affect the sensitivity and range of assay.
- Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat two times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- It is recommended that all standards and samples be assayed in duplicate
- It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results

5. REAGENT SUPPLIED

Kit Components	State	Specification
Capture Antibody	lyophilized	Polyclonal antibody
Detection Antibody	lyophilized	Biotinylated polyclonal antibody
Streptavidin-HRP Conjugate Conc.	concentrated	Streptavidin-HRP
Master Standard	lyophilized	Recombinant protein

6. MATERIAL REQUIRED BUT NOT SUPPLIED

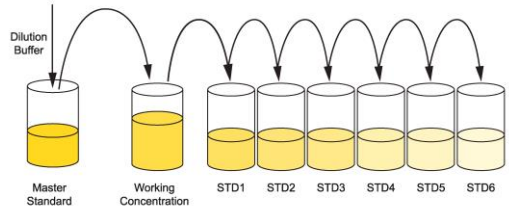
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)
- Multichannel pipette to deliver 100 µl with disposable tips
- Tubes for preparation standard dilutions
- Deionized (distilled) water
- Microwell plates (Corning Costar, Nunc Maxisorp)
- Coating Buffer: Phosphate-Buffered Saline (PBS)-11.36 g NA_2HPO_4, 2.40 g NAH_2PO_4, 5.84 g NaCl, add deionized water to 1l, pH=7.2.
- Dilution Buffer, Blocking Buffer: PBS+1% BSA
- Wash Solution: PBS+0.05% Tween
- Substrate Solution:TMB
- Stop Solution: 0.2 M H_2SO_4
- **We recommend the use BioVendor original components, see www.biovendor.com**

7. PREPARATION OF REAGENTS

- **All reagents need to be brought to room temperature prior to use**
- **Always prepare only the appropriate quantity of reagents for your test**
- **Do not use components after the expiration date marked on their label**

Capture Antibody
Reconstitute the lyophilized Capture Antibody with 1 ml of Coating Buffer (PBS) to obtain a stock solution with concentration 120 µg/ml. Dilute with PBS to a recommended final coating concentration of 2.0 µg/ml.
Detection Antibody
Reconstitute the lyophilized Detection Antibody with 0.6 ml of Dilution Buffer. Dilute 100x with Dilution Buffer to a recommended final concentration of 0.3 µg/ml.
Streptavidin-HRP Conjugate Conc.
Dilute Streptavidin-HRP Conjugate Conc. 200x with Dilution Buffer. Do not freeze!
Mouse Adiponectin Master Standard
Reconstitute the lyophilized Adiponectin Mouse HEK293 with 0.5 ml of Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (do not foam). The resulting concentration of mouse adiponectin in the Master Standard stock solution is 25 µg/ml.

A six point standard curve using 2-fold serial dilutions in Dilution Buffer starting at 8 ng/ml is recommended. Do not repeatedly freeze/thaw the Master Standard as loss of activity may occur.



8. PREPARATION OF SAMPLES

Attention: Choosing an appropriate diluent with respect to the given type of sample is indispensable for achieving satisfactory test performance. Dilution Buffer can affect absorbance as well as resulting concentration values. An appropriate dilution and a suitable diluent should be assessed by the researcher in advance to batch measurement
Recommended starting dilution for serum and plasma is 10000x.

9. PLATE PREPARATION

1. One day prior to running the test, prepare a coated microplate strips in the amount needed. Dilute the Capture Antibody to the working concentration in Coating Buffer. Let it dissolve at least 15 minutes and mix well (not to foam). Pipet **100 µl** of coating solution per well. Cover the plate and incubate overnight **(16-24 h)** at 2-8°C.
2. Aspirate and wash the wells once with Wash Solution (e.g. PBS+0.05% Tween, (0.35 ml per well). Invert and tap the plate strongly against a paper towel.
3. Block the wells by adding **200 µl** of Blocking Buffer per well. Incubate the plate at room temperature for **1 hour**. Aspirate and wash as in step 2. The plate is now ready for use or you can dry the plate overnight in an upside-down position at room temperature.

10. ASSAY PROCEDURE

1. Pipet **100 µl** of standards, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells
2. Incubate the plate at room temperature for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
4. Pipet **100 µl** of Detection Antibody solution into each well.
5. Incubate the plate at room temperature for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is less than 20°C. Do not shake with the plate during the incubation.
12. Stop the colour development by adding 100 µl of Stop Solution. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 11.

Note: Optimum duration of the incubation with the Substrate Solution depends on particular conditions of the test.

11. CALCULATIONS AND TYPICAL DATA

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of leptin ng/ml in samples. **The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor.**

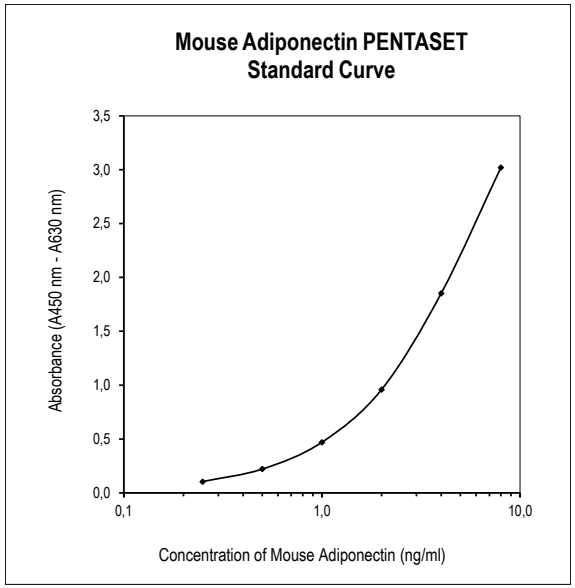


Figure 1: Expected Standard Curve for Mouse Adiponectin PENTASET.

- **Specificity**
  The antibodies used in this assay are specific for mouse adiponectin.

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MOUSE ADIPONECTIN PENTASET

Product Data Sheet

Cat. No.: RD220023

For Research Use Only

1. INTENDED USE

The BioVendor PENTASET is a development kit containing specific components including pre-optimized antibody matched pair, recombinant protein standard and streptavidin-HRP required for the development of a sandwich ELISA. Basic set is intended to perform assay on 5 plates. Use the recommended assay protocol, coating, blocking and dilution buffers, microwell plates, substrate and stop solutions to obtain required assay results.

- The inserted Product Data Sheet must be read before using this product
- The kit is intended for experienced ELISA users
- Store the kit at -20°C after the delivery. Under these conditions,the kit is stable until the expiration date (see label on the box).

2. INTRODUCTION

Adiponectin (244 aa) is expressed in adipose cells. It belongs to the soluble defence collagen superfamily containing collagen VIII a X homologous domain and C1q-like globular domain. Adiponectin forms covalently bound trimers (LMW), 2 trimers form hexamers (MMW) and their multiples (HMW) circulating in blood. The receptors AdipoR1 and AdipoR2 have been identified. Adiponectin increases insulin sensitivity and decreases plasma glucose by increasing tissue fat oxidation. It inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages. This adipokine plays a role as a scaffold of newly formed collagen in myocardial remodeling after ischaemic injury. Adiponectin has become a clinically relevant parameter to