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# **ab99968 – Adiponectin Human ELISA Kit**

For the quantitative measurement of Human Adiponectin in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

Abcam's Human Adiponectin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human Adiponectin in serum, plasma, and cell culture supernatants.

This assay employs an antibody specific for Human Adiponectin coated on a 96-well plate. Standards and samples are pipetted into the wells and Adiponectin present in a sample is bound to the wells by the immobilized antibody. The wells are washed, and biotinylated anti-Human Adiponectin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Adiponectin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add standard or sample to each well used. Incubate at room temperature.



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

### 4. Storage and Stability

**Store kit at -20°C immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

<b>Item</b>	<b>Amount</b>	<b>Storage Condition (Before Preparation)</b>
Adiponectin Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer	25 mL	-20°C
Recombinant Human Adiponectin Standard	2 vials	-20°C
Assay Diluent A	2 x 30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Assay Diluent C	2 x 15mL	-20°C
Biotinylated anti-Human Adiponectin	2 vial	-20°C
80X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2  $\mu$ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

## 8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.

- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

## 9. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

### 9.2 1X Wash Solution

If the 20X Wash buffer contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Solution.

### 9.3 1X Biotinylated Adiponectin Detection Antibody

Briefly spin the Biotinylated anti-Human Adiponectin vial before use. Add 100  $\mu$ L of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B a prior to use in the Assay Procedure.

### 9.4 1X HRP-Streptavidin Solution

Briefly spin the 80X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 80-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 100  $\mu$ L of 80X HRP-Streptavidin concentrate into a tube with 8 mL 1X Assay Diluent B to prepare a final 80 fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

## 10. Standard Preparation

- Prepare serially diluted standards immediately prior to use.
  - Always prepare a fresh set of standards for every use.
  - Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.
- 10.1** Briefly spin the vial of Adiponectin Standard. Prepare the 50 ng/mL Adiponectin **Stock Standard** by adding 800 µL 1X Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent C (for cell culture supernatants) into the vial (see table below).
- 10.2** Ensure the powder is thoroughly dissolved by gentle mixing.
- 10.3** Label tubes #1-7.
- 10.4** Prepare **Standard #1** by adding 180 µL 50 ng/mL Stock Standard to 320 µL Assay Diluent A or 1X Assay Diluent C into tube #1. Mix thoroughly and gently.
- 10.5** Pipette 400 µL Assay Diluent A or 1X Assay Diluent C into remaining tubes.
- 10.6** Prepare **Standard #2** by transferring 200 µL from tube #1 to #2, mix thoroughly.
- 10.7** Prepare **Standard #3** by transferring 200 µL from tube #2 to #3, mix thoroughly.
- 10.8** Using the table below as a guide, prepare further serial dilutions.
- 10.9** Assay Diluent A or 1X Assay Diluent C serves as the zero standard, (0 ng/mL).
- 10.10** The 18,000 pg/mL standard in Assay Diluent A may be saturated, we recommend starting from 6,000 pg/mL for Assay Diluent A Standard curve.

<b>Standard #</b>	<b>Volume to Dilute (µL)</b>	<b>Volume Diluent (µL)</b>	<b>Total Volume (µL)</b>	<b>Starting Conc. (pg/mL)</b>	<b>Final Conc. (pg/mL)</b>
<b>1</b>	180	320	500	50,000	18,000
<b>2</b>	200	400	600	18,000	6,000
<b>3</b>	200	400	600	6,000	2,000
<b>4</b>	200	400	600	2,000	666.7
<b>5</b>	200	400	600	666.7	222.2
<b>6</b>	200	400	600	222.2	74.07
<b>7</b>	200	400	600	74.07	24.69
<b>8</b>	0	400	400	0	0

## 11. Sample Preparation

### General Sample Information:

- If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent C should be used for dilution of culture supernatants.
- Suggested dilution for normal serum/plasma: 30,000 fold.
- For example, add 2  $\mu\text{L}$  of serum/plasma into a tube with 398  $\mu\text{L}$  Assay Diluent A to prepare a 200-fold diluted sample. Mix through and then pipette 2  $\mu\text{L}$  of prepared 200-fold diluted sample into a tube with 298  $\mu\text{L}$  1X Assay Diluent A to prepare a final 30,000 fold diluted sample.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

## 12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

## 13. Assay Procedure

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
  - **It is recommended to assay all standards, controls and samples in duplicate.**
- 13.1. Add 100  $\mu$ L of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
  - 13.2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300  $\mu$ L) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.
  - 13.3. Add 100  $\mu$ L of 1X Biotinylated Adiponectin Detection Antibody (Reagent Preparation section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.
  - 13.4. Discard the solution. Repeat the wash as in step 13.2.
  - 13.5. Add 100  $\mu$ L of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
  - 13.6. Discard the solution. Repeat the wash as in step 13.2.
  - 13.7. Add 100  $\mu$ L of TMB One-Step Substrate Reagent to each well.
  - 13.8. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
  - 13.9. Add 50  $\mu$ L of Stop Solution to each well. Read at 450 nm immediately.

## 14. Calculations

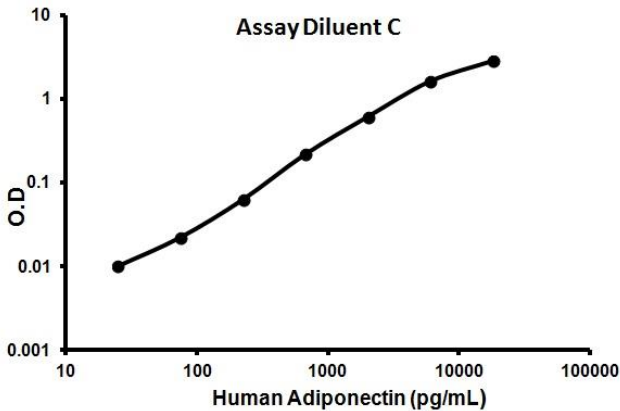
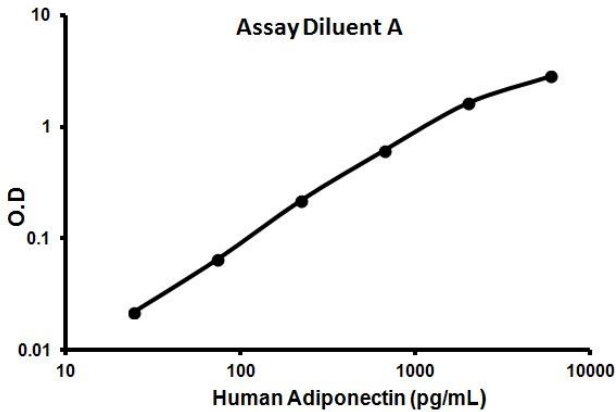
- 14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- 14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

**Δ Note:** *Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four- parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4- parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.*

- 14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- 14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

## 15. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.	
	Assay Diluent A	Assay Diluent C
24.69	0.022	0.01
74.07	0.065	0.022
222.2	0.219	0.062
666.7	0.614	0.216
2,000	1.621	0.604
6,000	2.832	1.601
18,000	-	2.802

## 16. Typical Sample Values

### SENSITIVITY –

The minimum detectable dose of Adiponectin is typically less than 25 pg/mL.

### RECOVERY –

Recovery was determined by spiking Human Adiponectin into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	93.57	83-104
Plasma	90.29	80-103
Cell Culture Media	96.59	83-107

## LINEARITY OF DILUTION –

Serum Dilution	Average % Expected Value	Range (%)
1:2	95	83-104
1:4	93	84-104

Plasma Dilution	Average % Expected Value	Range (%)
1:2	94	84-103
1:4	92	83-102

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	92	82-103
1:4	93	85-104

## PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	<10%	<12%

## 17. Assay Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- $\gamma$ , Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 $\alpha$ , MIP-1  $\beta$ , MIP-1 $\delta$ , PARC, PDGF, RANTES, SCF, TARC, TGF- $\beta$ , TIMP-1, TIMP-2, TNF- $\alpha$ , TNF- $\beta$ , TPO, VEGF.

## 18. Troubleshooting

Problem	Reason	Solution
<b>Low Precision</b>	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
<b>Precipitate in Diluent</b>	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
<b>Poor standard curve</b>	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
<b>Low Signal</b>	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
<b>Large CV</b>	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
<b>Low sensitivity</b>	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

# 19. Notes

## Technical Support

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