## Performance characteristics, continued

### Intra-assay precision

Four serum samples of known Hu adiponectin concentration were assayed in replicates of 5 to determine precision within an assay.

| Sample | Average (µg/mL) | SD | %CV |
|---|---|---|---|
| 1 | 1.86 | 0.07 | 3.82 |
| 2 | 5.90 | 0.23 | 3.84 |
| 3 | 8.50 | 0.28 | 3.31 |
| 4 | 23.36 | 0.69 | 2.97 |

SD = Standard Deviation; CV = Coefficient of Variation

Five urine samples of known concentrations of Hu adiponectin were assayed in replicates of 6 to determine precision within an assay.

| Sample | Average (ng/mL) | SD | %CV |
|---|---|---|---|
| 1 | 30.40 | 2.53 | 8.31 |
| 2 | 5.42 | 0.41 | 7.54 |
| 3 | 10.28 | 0.34 | 3.33 |
| 4 | 95.83 | 3.38 | 3.53 |
| 5 | 69.94 | 1.48 | 2.12 |

### Inter-assay precision

Four serum samples of known Hu adiponectin concentration were assayed in replicates of 5 to determine precision between assays.

| Sample | Average (µg/mL) | SD | %CV |
|---|---|---|---|
| 1 | 2.50 | 0.13 | 5.15 |
| 2 | 7.78 | 0.43 | 5.50 |
| 3 | 11.10 | 0.44 | 3.97 |
| 4 | 24.82 | 0.70 | 2.84 |

SD = Standard Deviation; CV = Coefficient of Variation

Five urine samples of known concentrations of Hu adiponectin were assayed in replicates of 3 to determine precision between assays.

| Sample | Average (ng/mL) | SD | %CV |
|---|---|---|---|
| 1 | 28.16 | 1.81 | 6.44 |
| 2 | 5.94 | 0.23 | 3.93 |
| 3 | 3.91 | 0.36 | 9.09 |
| 4 | 108.91 | 8.26 | 7.58 |
| 5 | 76.13 | 7.38 | 9.69 |

### Recovery

The recovery of adiponectin added to four different levels in five different serum samples and four different urine samples was measured with the Hu Adiponectin ELISA Kit.

| Type | Sample | Average % Recovery | % Range |
|---|---|---|---|
| Serum | 1 | 99.6 | 96–105 |
| | 2 | 99.8 | 96–104 |
| | 3 | 100.2 | 97–102 |
| | 4 | 92.5 | 88–95 |
| | 5 | 91.8 | 86–100 |
| Urine | 1 | 101.9 | 96-105 |
| | 2 | 97.9 | 96-104 |
| | 3 | 91.2 | 88-95 |
| | 4 | 84.7 | 80-90 |

### Linearity of Dilution

Different human serum samples containing adiponectin were diluted several fold (1:1,000 to 1:4,000).

| Samples | Sample Dilution | Expected (µg/mL) | Observed (µg/mL) | % of Expected |
|---|---|---|---|---|
| 1 | 1:1,000 | 13.61 | 13.61 | 100 |
| | 1:2,000 | 6.81 | 6.64 | 97.6 |
| | 1:4,000 | 3.40 | 2.97 | 87.2 |
| 2 | 1:1,000 | 15.89 | 15.89 | 100 |
| | 1:2,000 | 7.94 | 8.09 | 101.9 |
| | 1:4,000 | 3.97 | 3.76 | 94.7 |
| 3 | 1:1,000 | 11.51 | 11.51 | 100 |
| | 1:2,000 | 5.76 | 5.77 | 100.2 |
| | 1:4,000 | 2.88 | 2.51 | 87.1 |

Different human urine samples containing adiponectin were diluted several fold (1:5 to 1:10).

| Samples | Sample Dilution | Expected (ng/mL) | Observed (ng/mL) | % of Expected |
|---|---|---|---|---|
| 1 | 1:5 | 3.85 | 3.85 | 100 |
| | 1:10 | 1.92 | 2.00 | 104.2 |
| 2 | 1:5 | 5.14 | 5.14 | 100 |
| | 1:10 | 2.57 | 2.66 | 103.8 |
| 3 | 1:5 | 45.79 | 45.79 | 100 |
| | 1:10 | 22.89 | 23.56 | 102.9 |

### Expected values

Adiponectin levels in plasma and serum range from 4 to >15 µg/mL (from healthy donors).

Adiponectin levels in urine range from 3 to >15 ng/mL (from healthy donors).

### Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

### Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

### Product label explanation of symbols and warnings

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| REF | Catalog Number | LOT | Batch code | | Temperature limitation | | Use by | | Manufacturer | | Consult instructions for use | | Caution, consult accompanying documents |



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29 March 2016



# Human Adiponectin ELISA Kit

**Catalog. no.** KHP0041

**Pub. No.** MAN0005248 **Rev** 4.0

## Description

The Human Adiponectin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human adiponectin in serum, plasma, urine and cell culture supernatants. The assay will recognize both natural and recombinant human adiponectin.

Adiponectin is an adipocyte-specific protein and represents a major serum protein. The full length adiponectin in plasma exists as trimer, hexamer, and multimer. Extremely low amounts of the globular domain also exist in plasma as trimer.

## Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

| Components | Cat. No. KHP0041<br>96 tests |
|---|---|
| Hu Adiponectin Antibody Coated Wells. 96 well plate. | 6 × 16-well strip |
| Hu Adiponectin Standard. Lyophilized (64.0 ng recombinant Hu adiponectin). | 1 vial |
| Wash Buffer 10X. | 2 x 30 mL |
| ELISA Buffer 10X. | 2 x 30 mL |
| Detection Antibody. | 30 µL |
| HRP 100X (HRP Conjugated anti-rabbit IgG). | 150 µL |
| TMB Substrate Solution. | 12 mL |
| Stop Solution. | 12 mL |
| Adhesive Plate Covers. | 2 each |
| | |

**CAUTION!** This kit contains materials with small quantities of Proclin™ 300. Proclin 300 is toxic, corrosive, and a skin irritant, so avoid ingestion and contact with eyes, skin and mucous membranes. Observe all federal, state, and local regulations for disposal.

## Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

## General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- **Allow reagents to reach room temperature before use.** Mix to redissolve any precipitated salts.

**For Research Use Only. Not for use in diagnostic procedures.**

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

## Prepare 1X Wash Buffer

1. Dilute 10X Wash Buffer 1:10 (e.g., 30 mL of 10X Wash Buffer with 270 mL of deionized or distilled water). Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

## Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

## Dilute samples

Dilute **urine** samples 10-fold.

Dilute **serum** and **plasma** samples 2000-fold as follows:

1. Dilute 10 µL of serum with 990 µL of 1X ELISA Buffer (1:100 dilution). Mix well.
2. Dilute 50 µL of serum with 950 µL of 1:100 diluted serum (1:20 dilution).

Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

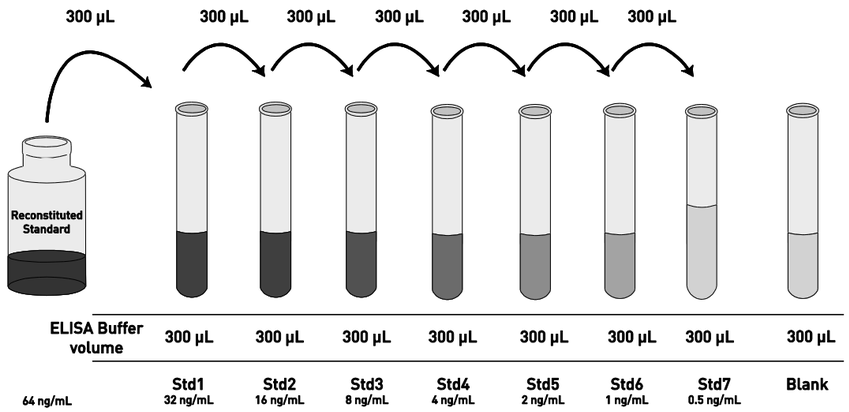
## Prepare 1X ELISA Buffer

Dilute 10X ELISA Buffer 1:10 (e.g., 20 mL 10X ELISA Buffer with 180 mL of deionized or distilled water). Label as 1X ELISA Buffer.

## Dilute standards

**Note:** Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu Adiponectin Standard with 1 mL of deionized water. Swirl or mix gently and allow the contents to sit for at least 15 minutes to ensure complete reconstitution. Label as 64 ng/mL Hu adiponectin.
2. Add 300 µL 1X ELISA Buffer to each of 8 tubes labeled as follows: 32, 16, 8, 4, 2, 1, 0. 5, and 0 ng/mL Hu adiponectin.
3. Make serial dilutions of the standard as shown in the dilution diagram below. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard.




## Prepare 1X Detection Antibody solution

Dilute Detection Antibody 1:1000 in 1X ELISA Buffer. Add 10 µL of Detection Antibody with 10 mL 1X ELISA Buffer. Label as 1X Detector Antibody.
**Diluted Detection Antibody is not stable and cannot be stored.**

## Prepare 1X HRP solution

Dilute HRP 100X 1:100. Add 100 µL with 10 mL of 1X ELISA Buffer. Label as 1X HRP solution.
**Use within 1 hour of preparation**.

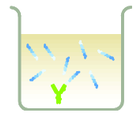


## ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3.5 hours.**

**IMPORTANT!** Perform a standard curve with each assay.

Determine the number of 16-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 4°C for up to 1 month.



### Bind antigen

1. Add 100 µL of the different standards in duplicate to the wells. Leave wells for chromogen blanks empty.
2. Add 100 µL of diluted serum, plasma, urine or cell culture supernatant samples (see page 2) to the appropriate wells.
3. Cover the plate with plate cover and incubate for 1 hour at 37°C.
4. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer (300 µL/wash).

### Add detector antibody

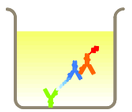
5. Add 100 µL Detection Antibody solution into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 1 hour at 37°C.
7. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer (300 µL/wash).

### Add Anti-rabbit IgG HRP

8. Add 100 µL 1X HRP solution into each well.
9. Cover the plate with plate cover and incubate for 1 hour at 37°C.
10. Thoroughly aspirate the solution and wash wells 5 times with 1X Wash Buffer (300 µL/wash).

### Add TMB substrate

11. Add 100 µL TMB Substrate Solution to each well. The substrate solution will begin to turn blue.
12. Incubate for 20 minutes at room temperature **in the dark**.
    **Note:** TMB should not touch aluminum foil or other metals.



### Add stop solution

13. Add 100 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.


## Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
1. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

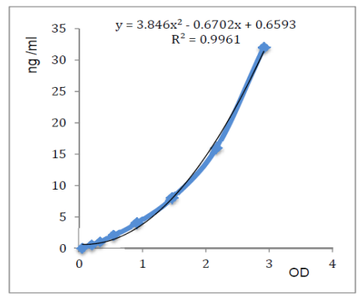
   **Note**: Dilute samples producing signals greater than that of the highest standard in 1X ELISA Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

## Performance characteristics

### Standard curve (example)

Typical standard curve over the range of 0–32 ng/mL Hu adiponectin.

| Standard Hu adiponectin (ng/mL) | Optical Density (450 nm) |
|---|---|
| 32 | 2.86 |
| 16 | 2.12 |
| 8 | 1.42 |
| 4 | 0.86 |
| 2 | 0.49 |
| 1 | 0.28 |
| 0.5 | 0.15 |
| 0 | 0 |




### Specificity

This kit is specific for the measurement of natural and recombinant human adiponectin. It does not cross-react with **Mouse** adiponectin, **Rat** adiponectin; **Human** resistin, RELM-β, leptin, TNF-α, or IL-6.

### Sensitivity

The minimum detectable dose of Hu adiponectin is 100 pg/mL.