Human Adiponectin ELISA Kit

Catalog Number KHP0041 (96 tests)

Pub. No. MAN0005248 **Rev.** 6.0 (30)

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

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human Adiponectin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is 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

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHP0041 (96 tests)
Hu Adiponectin Standard, lyophilized (64.0 ng recombinant Hu adiponectin)	1 vial
ELISA Buffer (10X)	2 x 30 mL
Antibody Coated Wells, 96-well plate	6 × 16-well strip
Hu Adiponectin Detection Antibody (1000X)	30 µL
Anti-Rabbit IgG HRP (100X)	150 µL
Wash Buffer Concentrate (10X)	2 x 30 mL
TMB Substrate Solution	12 mL
Stop Solution	12 mL
Adhesive Plate Covers	2 each

Materials required but not supplied

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

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 30 mL of Wash Buffer Concentrate (10X) 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

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- 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.

Prepare 1X ELISA Buffer

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

Pre-dilute samples

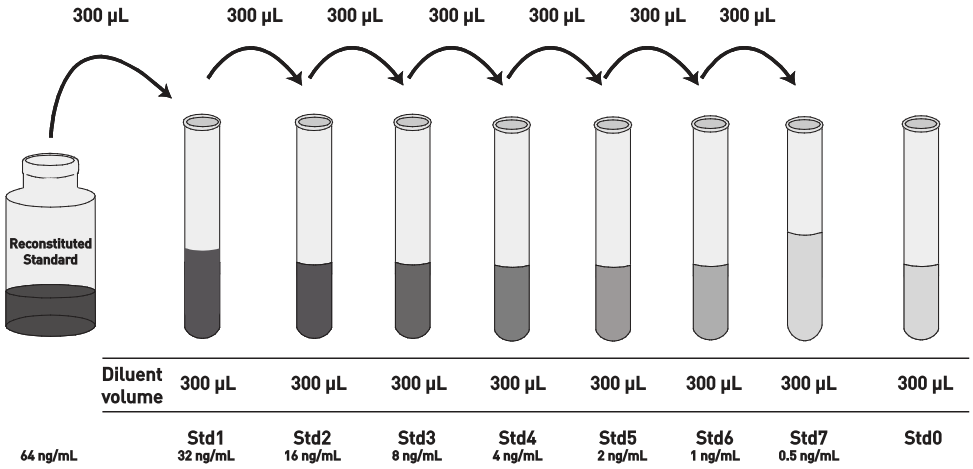
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with 1X ELISA Buffer.
- Dilute **urine** samples 10-fold.
- Dilute **serum** and **plasma** samples 2000-fold as follows:
  a. Dilute 10 µL of serum with 990 µL of 1X ELISA Buffer (1:100 dilution). Mix well.
  b. Dilute 50 µL 1:100 diluted serum with 950 µL of 1X ELISA Buffer (1:20 dilution).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu Adiponectin Standard to 64 ng/mL with 1 mL of deionized water. Swirl or mix gently and allow the contents to sit for 15 minutes to ensure complete reconstitution. Label as 64 ng/mL human adiponectin. **Use the standard within 1 hour of reconstitution.**
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 human adiponectin.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard.



Prepare 1X Detection Antibody solution

Dilute 10 µL of Detection Antibody (1000X) with 10 mL of 1X ELISA Buffer. Label as 1X Detector Antibody.

Note: The diluted Dectection Antibody is not stable and cannot be stored.

Prepare 1X Anti-Rabbit IgG HRP solution

Note: Use within 1 hour of preparation.

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of 1X ELISA Buffer. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 3.5 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate 1 hour at 37°C.
c. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

2 Add detector antibody

a. Add 100 µL of Hu Adiponectin Detection Antibody solution into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate 1 hour at 37°C.
c. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

3 Add IgG HRP

a. Add 100 µL Anti-Rabbit IgG HRP into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at 37°C.
c. Thoroughly aspirate the solution and wash wells 5 times with 1X Wash Buffer.

4 Add TMB Substrate Solution

a. Add 100 µL TMB Substrate Solution to each well. The substrate solution begins to turn blue.
b. Incubate for 20 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate 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 4 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.
3. 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.

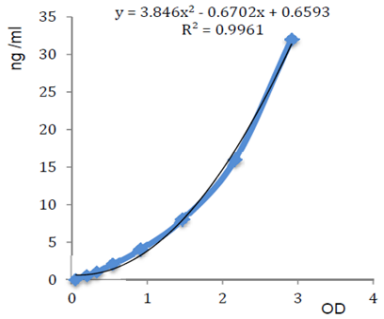
   Note: Dilute samples producing signals greater than the upper limit of the standard curve in 1X ELISA Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

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

Standard Human 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



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).

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/mL)	2.50	7.78	11.10	24.82
Standard Deviation	0.13	0.43	0.44	0.70
% Coefficient of Variation	5.15	5.50	3.97	2.84

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

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (ng/mL)	28.16	5.94	3.91	108.91	76.13
Standard Deviation	1.81	0.23	0.36	8.26	7.38
% Coefficient of Variation	6.44	3.93	9.09	7.58	9.69

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/mL)	1.86	5.90	8.50	23.36
Standard Deviation	0.07	0.23	0.28	0.69
% Coefficient of Variation	3.82	3.84	3.31	2.97

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

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (ng/mL)	30.40	5.42	10.28	95.83	69.94
Standard Deviation	2.53	0.41	0.34	3.38	1.48
% Coefficient of Variation	8.31	7.54	3.33	3.53	2.12

Sensitivity

The analytical sensitivity of this assay is 100 pg/mL Hu adiponectin.

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.

Linearity of dilution

Different human serum samples containing adiponectin were assayed at dilutions from 1:1,000 to 1:4,000.

# of Samples	Sample Dilution	Expected (µg/mL)	Observed (µg/mL)	% 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 assayed at 1:5 and 1:10 dilutions.

# of Samples	Sample Dilution	Expected (ng/mL)	Observed (ng/mL)	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

Recovery

The recovery of human adiponectin added to four different levels in five different serum samples and four different urine samples was measured with the Human 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

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.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

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.