Rat Adiponectin ELISA Kit

Catalog. no. KRP0041

Pub. No. MAN0003486 **Rev** 4.0

Description

The Rat Adiponectin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of rat adiponectin in serum, plasma, and cell culture supernatants. The assay will recognize both natural and recombinant rat 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. KRP0041 96 tests
Rat Adiponectin Antibody Coated Wells. 96 well strip-well plate.	1 plate
Rat Adiponectin Standard. Lyophilized	1 vial
Wash Buffer 10X.	2 × 30 mL
ELISA Buffer 10X.	2 × 30 mL
Detection Antibody.	20 µL
HRP 100X (HRP Conjugated anti-mouse IgG).	150 µL
TMB Substrate Solution.	12 mL
Stop Solution.	12 mL
Adhesive Plate Covers.	2

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

- 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.

Prepare 1X Wash Buffer

1. Dilute 10X Wash Buffer 1:10 (e.g., 50 mL of 10X Wash Buffer with 450 mL of deionized 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 **serum** and **plasma** samples 1,000-fold.

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

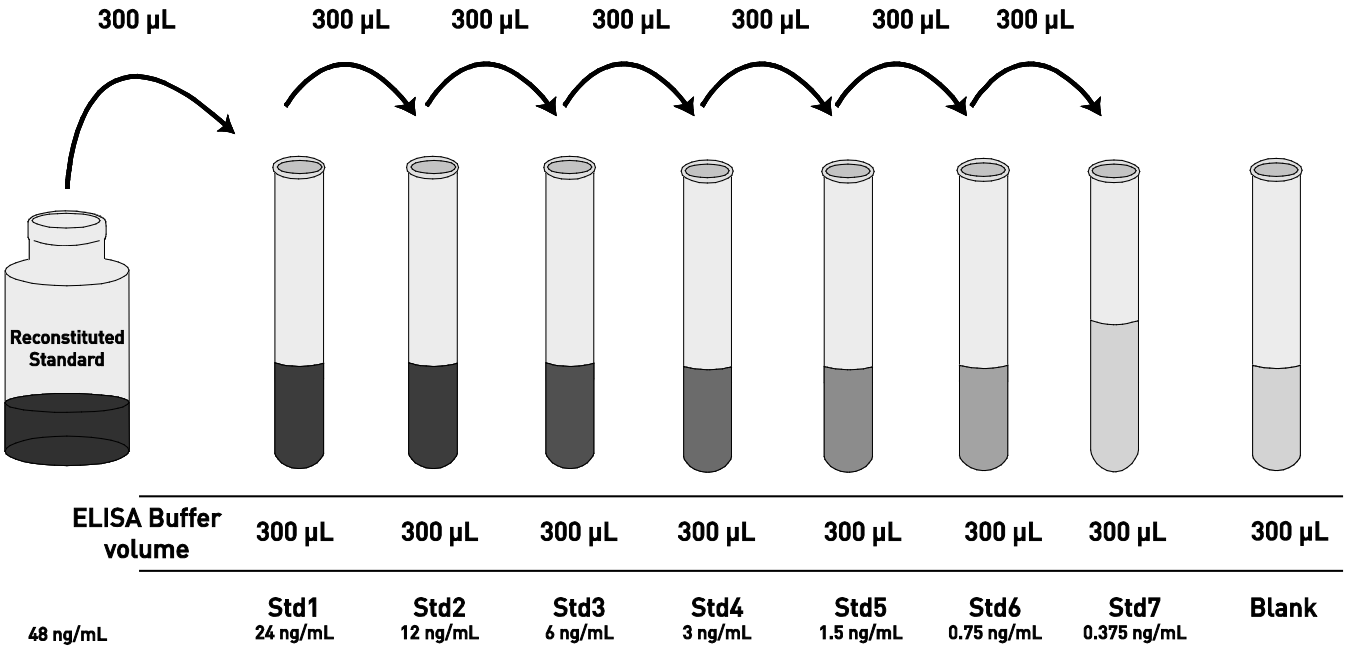
Prepare 1X ELISA Buffer

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

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rat 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 48 ng/mL Rat adiponectin.
2. Add 300 µL 1X ELISA Buffer to each of 8 tubes labeled as follows: 24, 12, 6, 3, 1.5, 0.75, 0.375, and 0 ng/mL Rat 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 1:1,000 in 1X ELISA Buffer. Add 10 µL of Detection Antibody with 10 mL 1X ELISA Buffer. Label as 1X Detector Antibody.

The diluted Detection Antibody is not stable and cannot be stored.

Prepare 1X HRP solution

Dilute HRP 100X 1:100. Add 100 µL to 10 mL of 1X ELISA Buffer. Label as 1X HRP solution.

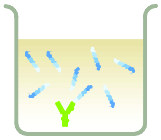
Use within 1 hour of preparation.

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.

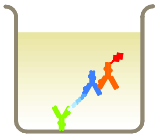


Bind antigen

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, 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).

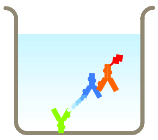
Add detector antibody

5. Add 100 µL 1X 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-mouse IgG HRP

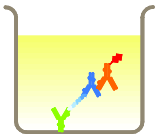
8. Add 100 µL 1X HRP solution into each well.
9. Cover the plate with plate cover and incubate for 1 hour at 37°C.
10. Thoroughly aspirate the solution and wash wells 5 times with 1X Wash Buffer (300 µL/wash).



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.
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.

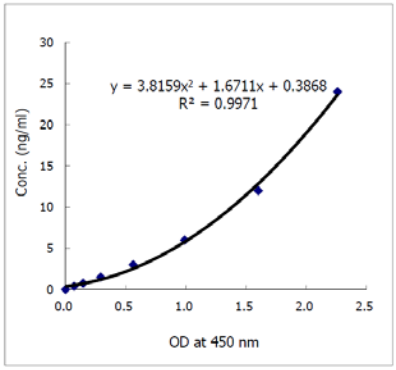
   Note: Dilute samples producing signals greater than that of the highest standard in 1X ELISA Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

Typical standard curve over the range of 0–24 ng/mL Rat adiponectin.

Standard Rat adiponectin (ng/mL)	Optical Density (450 nm)
24	2.26
12	1.60
6	0.99
3	0.56
1.5	0.29
0.75	0.15
0.375	0.07
0	0



Specificity

This ELISA is specific for the measurement of natural and recombinant rat adiponectin. It does not cross-react with **Human** adiponectin or TNF-α; **Rat** Nampt, resistin, RELM-α, or leptin; **Mouse** adiponectin.

Sensitivity

The minimum detectable dose of Rat adiponectin is 50 pg/mL.

Performance characteristics, continued

Intra-assay precision

Eight samples of known Rat adiponectin concentration were assayed in replicates of 12 to determine precision within an assay.

Sample	Average (µg/mL)	SD	%CV
1	7.70	0.18	2.29
2	6.04	0.37	6.07
3	10.10	0.50	4.96
4	11.61	0.75	6.48
5	11.89	0.26	2.19
6	4.41	0.33	7.53
7	6.29	0.22	3.43
8	4.67	0.25	5.40

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

Eight samples of known Rat adiponectin concentration were assayed in replicates of 8 to determine precision between assays.

Sample	Average (µg/mL)	SD	%CV
1	5.45	0.30	5.55
2	8.04	0.49	6.08
3	4.20	0.11	2.60
4	7.78	0.34	4.42
5	9.04	0.48	5.35
6	7.80	0.28	3.60
7	3.25	0.10	3.21
8	2.49	0.20	8.10

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

When serum or plasma samples are spiked with known concentrations of rat adiponectin, the recovery averages 95% (range 87-105%).

Sample	Average % Recovery	% Range
1	97.6	96-100
2	95.1	87-104
3	97.7	94-103
4	96.8	91-100
5	95.1	92-97
6	93.7	88-97
7	94.0	91-97
8	93.5	92-97

Linearity of Dilution

Different rat serum samples containing adiponectin were diluted several fold (1:1,000 to 1:2,000).

Samples	Sample Dilution	Expected (µg/mL)	Observed (µg/mL)	% of Expected
1	1:1,000	12.9	12.9	100.0
	1:1,200	10.8	11.0	102.3
	1:1,500	8.6	8.5	98.6
	1:1,700	7.6	7.7	100.6
	1:2,000	6.5	5.8	90.0
2	1:1,000	6.2	6.2	100.0
	1:1,200	5.2	5.2	100.1
	1:1,500	4.1	4.1	99.5
	1:1,700	3.6	3.6	98.9
	1:2,000	3.1	3.0	96.9

Expected values

Adiponectin levels in plasma and serum range from 3 to >7 µg/mL (from normal rats).

Limited product warranty

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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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