Mouse Adiponectin ELISA Kit

Catalog. no. KMP0041

Pub. No. MAN0004522 **Rev** 5.0

Description

The Mouse Adiponectin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of mouse adiponectin in serum, plasma, and cell culture supernatants. The assay will recognize both natural and recombinant mouse 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. KMP0041 96 tests
Ms Adiponectin Antibody Coated Wells. 96 well strip plate.	1 plate
Ms Adiponectin Standard. Lyophilized.	1 vial
Wash Buffer 10X.	2 × 30 mL
ELISA Buffer 10X.	2 × 30 mL
Detection Antibody.	60 µL
HRP 100X (HRP Conjugated anti-rabbit 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 20,000-fold.

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

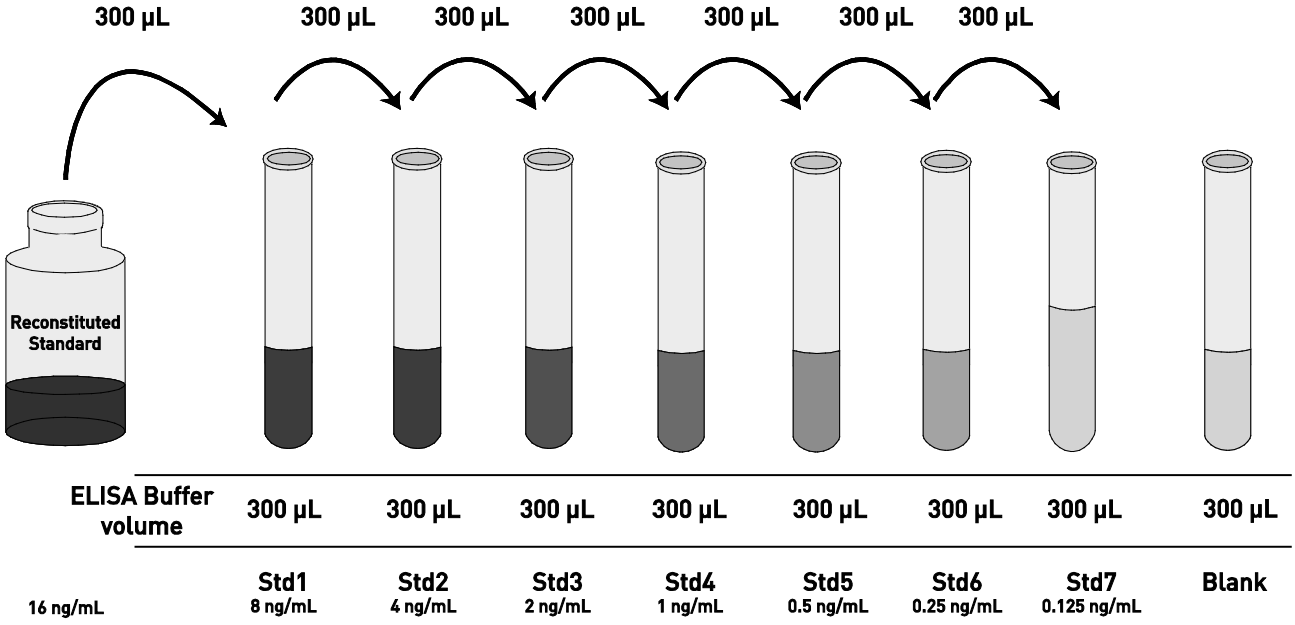
Prepare 1X ELISA Buffer

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 Ms Adiponectin Standard with 1 mL of deionized water. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 16 ng/mL Ms adiponectin.
2. Add 300 µL 1X ELISA Buffer to each of 8 tubes labeled as follows: 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0 ng/mL Ms 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 1:200 in 1X ELISA Buffer. Add 50 µL of Detection Antibody to 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.

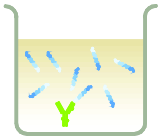
Use within 1 hour of preparation.

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.

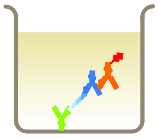


Bind antigen

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).

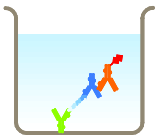
Add detector antibody

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).



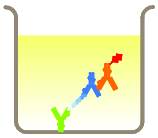
Add Anti-rabbit IgG HRP

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

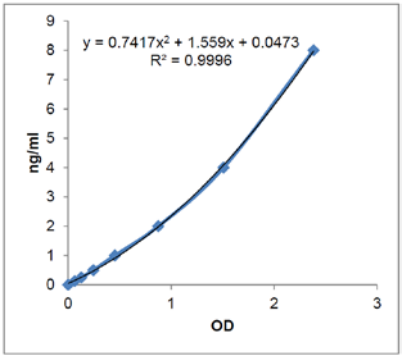
1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
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 0–8 ng/mL Ms adiponectin.

Standard Ms adiponectin (ng/mL)	Optical Density (450 nm)
8	2.38
4	1.51
2	0.88
1	0.45
0.5	0.24
0.25	0.13
0.125	0.06
0	0



Specificity

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

Sensitivity

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

Performance characteristics, continued

Intra-assay precision

Five samples of known Ms adiponectin concentration were assayed in replicates of 10 to determine precision within an assay.

Sample	Average (µg/mL)	SD	%CV
1	19.16	0.37	1.91
2	15.52	0.64	4.15
3	23.99	0.35	1.44
4	17.62	0.43	2.45
5	17.19	0.56	3.23

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

When serum samples are spiked with known concentrations of mouse adiponectin, the recovery averages 97% (range 91-105%).

Sample	Average % Recovery	% Range
1	102.2	98-105
2	96.0	93-98
3	96.0	91-98
4	95.0	92-97
5	98.0	93-102

Expected values

Adiponectin levels in plasma and serum range from 10 to >80 µg/mL (from normal mice).

Inter-assay precision

Five samples of known Ms adiponectin concentration were assayed in replicates of 10 to determine precision between assays.

Sample	Average (µg/mL)	SD	%CV
1	18.01	0.49	2.71
2	15.07	1.00	6.62
3	18.78	0.79	4.19
4	15.95	1.26	7.88
5	16.17	0.78	4.80

SD = Standard Deviation; CV = Coefficient of Variation

Linearity of Dilution

Different mouse serum samples containing adiponectin were diluted several fold (1:20,000 to 1:40,000).

Samples	Sample Dilution	Expected (µg/mL)	Observed (µg/mL)	% of Expected
1	1:20,000	33.18	33.18	100.0
	1:30,000	22.12	24.44	110.5
	1:40,000	16.59	17.06	102.8
2	1:20,000	40.47	40.47	100.0
	1:30,000	26.98	26.94	99.8
	1:40,000	20.24	21.75	107.5
3	1:20,000	52.48	52.48	100.0
	1:30,000	34.98	32.88	94.0
	1:40,000	26.24	26.92	102.6

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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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