ab285347 – Mouse Adiponectin ELISA Assay Kit

For quantitative determination of adiponectin in mouse serum, plasma or various tissue or cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285347

Storage and Stability

Reagents must be stored at 4°C when not in use. The reagents must be brought up to room temperature before use. Do not expose the reagents to temperature above 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Supplied

Item	Quantity	Storage Condition
Adiponectin antibody pre-coated Microplate	6 x 16 wells	4 °C
10X Wash concentrate	2 x 30 ml	4 °C
10X ELISA Buffer	2 x 30 ml	4 °C
Detection Antibody	60 µl	4 °C
100X HRP Conjugate	150 µl	4 °C
Standard (lyophilized)	16 ng	4 °C
TMB	12 ml	4 °C
Stop Solution	12 ml	4 °C
Plate sealers	2	4 °C

Reagent Preparation

- Allow all samples and kit components to equilibrate to room temperature (20 - 25°C).
- Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below).
- It is recommended that standards and samples be run in duplicate.

1X Wash Solution: Dilute 10X Wash Concentrate to 1X with deionized water. The diluted 1X Wash Solution is stable for one month at RT.

1X ELISA Buffer: Dilute 10X ELISA Buffer to 1X with deionized water.

HRP Conjugate: Dilute 100X HRP Conjugate to 1X with 1X ELISA Buffer. Use the 1X HRP Conjugate within one hour of preparation.

1X Detection Antibody: Dilute 1:200 in 1X ELISA Buffer. Use within 1 hour.

Standard Preparation

1. Briefly centrifuge the lyophilized Standard vial.
2. When opening, remove cap gently as the lyophilizate may have become dislodged during shipping.
3. Add 1 ml of deionized water to make a stock concentration of 16 ng/ml. Mix well.
4. Aliquot and store at - 20°C for future use. A recommended dilution scheme is as follows:
5. Label 8 microcentrifuge tubes #0 – 7 and add 300 µl 1x ELISA Buffer to each microcentrifuge tube.
6. Add 300 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 8 ng/ml.
7. Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0.

Tube no.	Stock	7	6	5	4	3	2	1	0
Conc (ng/ml)	16	8	4	2	1	0.5	0.25	0.125	0
1X ELISA Buffer (µl)	-	300	300	300	300	300	300	300	300

Sample Preparation

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

Serum, Plasma or Cell Culture Supernatant: Must be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.

Δ Note: *As a starting point, 1/20,000 dilution of serum or plasma are recommended! If samples values fall outside the detection range of the assay, a lower or higher dilution may be required!*

Assay Protocol

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µl of Standard 0 to 7 and 100 µl of diluted Samples into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3. Cover the plate with plate sealer and incubate at 37°C for 1 hr.
4. Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
5. Add 100 µl 1X Detection Antibody to each well.
6. Cover the plate with plate sealer and incubate at 37°C for 1 hr.
7. Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
8. Add 100 µl 1X HRP Conjugate to each well
9. Cover the plate with plate sealer and incubate at 37°C for 1 hr.
10. Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.
11. Add 100 µl of the TMB Solution to each well.
12. Incubate at room temperature for 20 min. Protect from light.
13. Using a multi-channel pipette, add 100 µl Stop Solution to each well.

Measurement

Read absorbance at 450 nm. Subtract the absorbance of the blank from the readings for each standard and sample.

Calculation

1. Construct the standard curve by plotting the known concentration (X) of standard versus the absorbance (Y) of standard
2. A typical linear range is shown between 0.125 ng/ml and 2 ng/ml.
3. Calculate the adiponectin concentrations of samples by interpolation of the quadratic regression curve formula.
4. The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted sample.

Technical Support

For all technical or commercial enquiries please go to:
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