RAT/MOUSE ADIPONECTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RAT OR MOUSE ADIPONECTIN CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

ELISA NAME	RAT/MOUSE ADIPONECTIN ELISA
Catalog No.	SK00010-06
Formulation	96 T
Lot No.	
Standard Range	3.2 – 2000 ng/mL
Sensitivity	3.2 ng/mL
Sample Volume	50 μL
Sample Pretreatment	Require
Sample Dilution	4 with pretreatment **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA Plasma
Specificity	Mouse and Rat Adiponectin
Calibration	Mouse Adiponectin Recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Rat/Mouse Adiponectin ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Adiponectin from serum and plasma in a competitive EIA format.

This immunoassay contains recombinant and biotinylated recombinant mouse Adiponectin, and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Adiponectin.

ASSAY OVERVIEW

Rat/Mouse Adiponectin ELISA employs the quantitatively competitive EIA format. Rat or Mouse Adiponectin present in samples compete with a fixed amount of biotinylated Adiponectin for sites on purified rabbit IgG specific against Adiponectin. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplate. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of rat or mouse Adiponectin bound in the initial step. The sample values are then read off the standard curve.

PROCEDURAL LIMITATIONS

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well polystyrene microplate coated with polyclonal antibody against rabbit IgG Fc	**RM01**	**1 plate**
Adiponectin Standard – 2000 ng/vial of recombinant mouse Adiponectin in a buffered protein base with preservative; lyophilized.	**010-06-01**	**1 vial**
Biotin Concentrate - 600 µL/vial, 10-fold concentrate of mouse Adiponectin biotinylated with preservative; lyophilized.	**010-06-02**	**1 vial**
Antibody Concentrate– 600 µL/vial, 10-fold concentrate of polyclonal antibody against mouse Adiponectin with preservative; lyophilized.	**010-06-03**	**1 vial**
Positive Control – one vial of recombinant mouse Adiponectin; lyophilized (optional).	**010-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Sample Buffer – 20 mL of 0.1% SDS solution.	**STB01**	**1 bottle**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB18**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**

Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standards, Positive Controls, Antibody Concentrate and Biotin Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Biotin concentrated solution and Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Reconstituted Biotin concentrated solution (350 µL) CAN NOT BE STORED at 2 – 8° C. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8° C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- PBS
- TCEP 500 mM, 400~ 500µl

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

*Samples need to be treated before being added to the microplate. Standard and Positive Control **DO NOT NEED** to be treated.*

1. Add 240 µl of 500 mM TCEP to 11.76 ml of Sample Buffer (STB01) to prepare the **Pretreatment Solution (10mM TCEP, 0.1% SDS).**
2. Add 50 µL of sample to 150 µl of **Pretreatment Solution** in a polypropylene tube. **Note:** This pretreatment dilution (4-fold dilution) may require optimization.
2. Vortex gently and incubate for 10-30 minutes at room temperature. Assay immediately and discard any excess pretreated samples.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

***Note:** Pretest is required to optimize the dilution factor needed for samples. If sample values are too high, then the sample should be diluted with **PBS** prior to pretreatment.

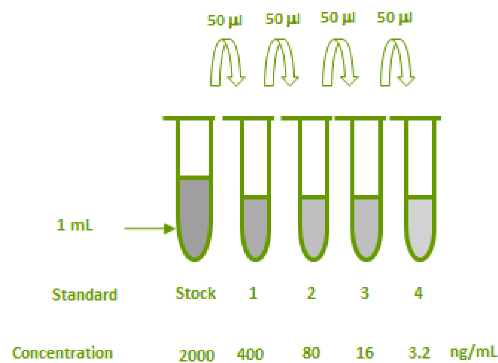
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Adiponectin Standard - Reconstitute the Adiponectin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	2000 ng/ml
# 1	50 µl of stock	200 µl	400 ng/ml
# 2	50 µl of 1	200 µl	80 ng/ml
# 3	50 µl of 2	200 µl	16 ng/ml
# 4	50 µl of 3	200 µl	3.2 ng/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Antibody Concentrate - Reconstitute with 600 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 5.4 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 600 µL of 10-fold concentrated stock solution to prepare working solution.

Biotin Solution - Reconstitute with 600 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 5.4 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 600 µL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** Streptavidin-HRP Conjugate working solution should be used within a few days. **Protect from light.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave two wells as Blank. **DO NOT add Antibody or Biotin Solution into Blank wells.**
4. Add 50 µL of Dilution Buffer to Total Binding wells. Add 50 µL per well of Standard dilutions to the appropriate wells. Add 50 µL of Positive Control into the appropriate wells. Add 50 µL of samples into appropriate wells.
5. Add 50 µL of Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. **Note: DO NOT ASPIRATE OR WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.**
6. Add 50 µL of Biotin working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to all wells (including Blank wells). Incubate for 60 min on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 7.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the standard concentrations versus the log of the O.D. and the

best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

ADIPONECTIN STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.075)
Total Binding	1.072
3.2	0.901
16	0.539
80	0.091
400	0.023
2000	0.021

SPECIFICITY

Mouse Adiponectin ELISA Kit recognizes recombinant and endogenous mouse Adiponectin. Data also indicates that rat samples can competitively bind to antibody that was used in this kit formulation. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. This means rat samples cross-react with Mouse Adiponectin ELISA kit.

PROTEINS	CROSS-REACTIVITY (%)
Mouse Adiponectin, Full Length	100
Mouse CTRP3, globular form	0
Mouse CTRP9, globular form	0
Mouse Leptin	0
Mouse Visfatin	0
Mouse FABP-4	0
Mouse FGF-21	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇓
Add 50 µl of standard dilutions, the pretreated samples, or positive control to each well. Add 50 µl of Antibody working solution to each well used. Incubate 2 hours on the plate shaker at RT. **DO NOT** add Antibody or Biotin Solution into Blank wells.
⇓
DO NOT ASPIRATE OR WASH PLATE. Add 50 µl Biotin working solution to each well used. Incubate 2 hours on the plate shaker at RT.
⇓
Aspirate and wash 4 times.
⇓
Add 100 µl Streptavidin-HRP conjugate to all wells, including blanks. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⇓
Aspirate and wash 4 times.
⇓
Add 100 µl Substrate Solution to each well. Incubate 5-15 min on the plate shaker at RT. **Protect from light.**
⇓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.