HUMAN ADIPONUTRIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ADIPONUTRIN CONCENTRATIONS IN CELL CULTURE OR TISSUES

PURCHASE INFORMATION:

ELISA NAME	HUMAN ADIPONUTRIN ELISA
Catalog No.	SK00067-02
Lot No.	
Formulation	96 T
Standard range	1.9-120 ng/mL
Sensitivity	0.2 ng/mL
Sample Volume	100 µl
Sample Type	Cell Culture or Tissues
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human ADIPONUTRIN only
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2°C - 8°C

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

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INTRODUCTION

Human ADIPONUTRIN immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human ADIPONUTRIN in cell culture or tissues. It contains recombinant human ADIPONUTRIN and antibodies raised against this protein. It has been shown to accurately quantify recombinant human ADIPONUTRIN. Results obtained with naturally occurring ADIPONUTRIN samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human ADIPONUTRIN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for ADIPONUTRIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ADIPONUTRIN present is bound by the immobilized antibody. After washing away any unbound substances, a rabbit polyclonal antibody specific for ADIPONUTRIN is added to the wells. Following a wash to remove any unbound antibody, a Goat anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of ADIPONUTRIN bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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 dilution buffer, 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Adiponutrin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against ADIPONUTRIN.	**067-02-01**	**1 plate**
Adiponutrin Standard – 600 ng/vial of recombinant human ADIPONUTRIN in a buffered protein base with preservatives; lyophilized.	**067-02-02**	**1 vial**
Detection Antibody Concentrate – 110 µL/vial, 100-fold concentrated of polyclonal antibody against Adiponutrin with preservatives; lyophilized.	**067-02-03**	**1 vial**
Positive Control - one vial of recombinant human Adiponutrin, lyophilized	**067-02-04**	**1 vial**
Goat Anti Rabbit IgG-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Goat Anti rabbit IgG HRP conjugate	**ARIGHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservatives	**DB07**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	**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 solution	**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 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Goat Anti Rabbit IgG-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care should be taken while handling this solution. We recommend that this product be handled by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

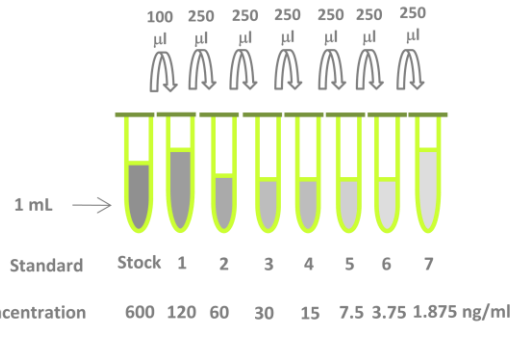
REAGENT PREPARATION

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 Wash Buffer.

ADIPONUTRIN Standard - Refer to vial label for reconstitution volume. Reconstitute the **ADIPONUTRIN** Standard with 1.0 ml of Dilution Buffer (**DB01**). This reconstitution produces a stock solution of 600 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 400 µL of Dilution Buffer into tube #1 and transfer 100 µL of the stock solution to produce the high concentration standard of 120 ng/mL. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use tube #1 working solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The 120 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	600 ng/ml
# 1	100 µl of stock	400 µl	120 ng/ml
# 2	250 µl of 1	250 µl	60 ng/ml
# 3	250 µl of 2	250 µl	30 ng/ml
# 4	250 µl of 3	250 µl	15 ng/ml
# 5	250 µl of 4	250 µl	7.5 ng/ml
# 6	250 µl of 5	250 µl	3.75 ng/ml
# 7	250 µl of 6	250 µl	1.87 ng/ml



Detection Antibody Concentrate - Reconstitute the **Detection Antibody Concentrate** with 110 µl of Antibody Diluent Solution (**DB07**) to produce a 100-fold concentrated stock solution. Pipette 10.89 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution.

Goat Anti Rabbit IgG-HRP Conjugate - Pipette 11.94 mL of HRP Diluent Solution (**DB08**) into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of ARIG-HRP should be used within a few days.

Positive Control - Reconstitute **Positive Control** with 1.0 mL of Dilution Buffer (**DB01**). **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous section.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer (**DB01**) to Blank wells (A2, A3).
4. Add 100 µL of Standard (from B2, B3 to G2, G3, and G4, G5), sample, or positive control (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Goat Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes 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 micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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 ADIPONUTRIN 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 standard curve should be generated for each set of samples assayed.

ADIPONUTRIN (NG/ML)	AVERAGE OD450 (CORRECTED)*
BLANK	0 (0.176)
0.938 (optional)	0.015
1.875	0.038
3.75	0.077
7.5	0.126
15	0.217
30	0.434
60	0.692
120	1.306

- Lot No.:
- Positive Control:

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant human ADIPONUTRIN.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of ADIPONUTRIN was 0.2 ng/mL.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Goat Anti Rabbit IgG HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-15 minutes on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Adiponutrin	100
ATGL	0
FTO	0
Endothelial Lipase	0
ADRP	0
NGAL	0
CTRP9	0
CTRP3	0
Omentin 1	0
Visfatin	0
FGF-21	0
RBP-4	0