HUMAN ADIPOSE TRIGLYCERIDE LIPASE (ATGL) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ADIPOSE TRIGLYCERIDE LIPASE (ATGL) CONCENTRATIONS IN CULTURE SUPERNATES AND TISSUE HOMOGENATES

PURCHASE INFORMATION:

ELISA NAME	HUMAN ATGL ELISA
Catalog No.	SK00576-01
Lot No.	
Formulation	96 T
Standard Range	0.78-100 ng/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Cell Culture Supernates and Tissue Homogenates
Specificity	Human ATGL
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2 – 8 °C

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INTRODUCTION

Human adipose triglyceride lipase (ATGL) immunoassay is a solid phase ELISA designed to measure human ATGL in cell culture supernates and tissue homogenates. It contains recombinant human ATGL and antibodies raised against this protein. It has been shown to accurately quantify recombinant human ATGL. Results obtained with naturally occurring ATGL 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 ATGL.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for ATGL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ATGL present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for ATGL is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP 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 ATGL 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 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Human ATGL Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against ATGL.	**576-01-01**	**1 plate**
ATGL Standard – 100 ng/vial of recombinant ATGL in a buffered protein base with preservative; lyophilized.	**576-01-02**	**1 vial**
Detection Antibody Concentrate – 600 µL/vial, 10-fold concentrate of biotinylated antibody against ATGL with preservative; lyophilized.	**576-01-03**	**2 vials**
Positive Control – one vial of recombinant human ATGL; lyophilized.	**576-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated Streptavidin-HRP conjugate solution with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB01**	**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 Standard, Positive Control and Detection Antibody Concentrate should

be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8 °C for up to 8 months. Do not freeze TMB substrate solution (**protect from light**).
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.

OTHER SUPPLIES 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate precautions should be taken while handling this solution. We therefore recommend that this product is handled only 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 PREPARATION

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

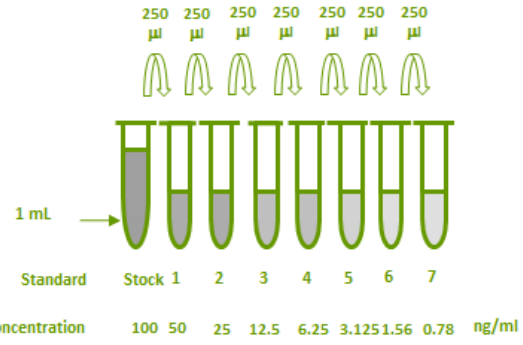
REAGENT PREPARATION

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.

ATGL Standard - Refer to vial label for reconstitution volume. Reconstitute the **ATGL** standard with 1.0 mL of **Dilution Buffer**. This reconstitution produces a stock solution of **100 ng/mL**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 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	1 ml	100 ng/ml
# 1	250µl of stock	250µl	50 ng/ml
# 2	250µl of 1	250µl	25 ng/ml
# 3	250µl of 2	250µl	12.5 ng/ml
# 4	250µl of 3	250µl	6.25 ng/ml
# 5	250µl of 4	250µl	3.125 ng/ml
# 6	250µl of 5	250µl	1.56 ng/ml
# 7	250µl of 6	250µl	0.78 ng/ml



Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 600 µL of **Dilution Buffer** to prepare a 10-fold concentrated solution. Pipette 5.4 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer the 600 µL of 10-fold concentrated solution to the tube to make 1x working solution. **Note**: This is enough for half of the plate, there are 2 vials of Detection Antibody Concentrate included in this kit.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge

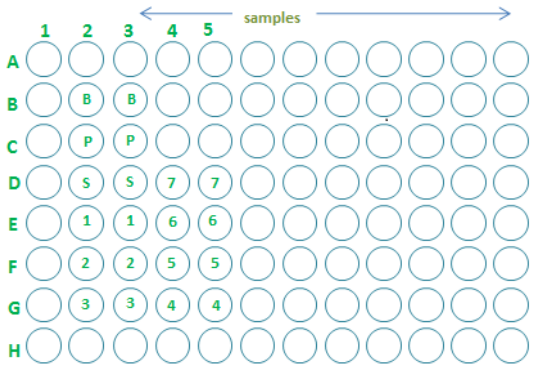
tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note**: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of **Dilution Buffer**. **Note**: Positive Control solution could be reused within a few days if stored at -20 °C or -70 °C.

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 sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch (P01) with the desiccant pack.
3. Add 100µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (D4, D5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate 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 1x Wash Buffer (300µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for 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 100µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate 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 5-10 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100µL 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 450nm.



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 ATGL 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.

Calculation of samples with a concentration exceeding that of standard 100 ng/ml may result in inaccurate, low human ATGL levels. Such samples require further external pre-dilution according to expected human ATGL values with Dilution Buffer in order to precisely quantify the actual human ATGL level.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human ATGL.

SENSITIVITY

The minimum detectable dose (MDD) of ATGL was 30 pg/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.081)
0.781	0.059
1.563	0.124
3.125	0.201
6.25	0.367
12.5	0.611
25	0.908
50	1.227
100	1.539

- Lot No.:
- Positive Control:

SPECIFICITY

Proteins	Crossreactivity (%)
Human ATGL	100
Mouse ATGL	0
Human Adiponutrin	0
Human Endothelial Lipase	0
Human Vaspin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓

Add 100 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 1 hour on plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate 5-10 min on plate shaker at RT. **Protect from light.**

↓

Add 100 µl Stop Solution to each well. Read at 450nm within 15 min.