Lipase Activity Assay Kit

Item No. 700640

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

For best results store this kit as supplied (-80°C), or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700003	Sodium Phosphate Assay Buffer	1 vial/10 ml	-20°C
700006	Fluorometric Thiol Detector	1 vial/300 µl	-20°C
700641	Lipase Substrate	1 vial/1.2 ml	-80°C
700642	Lipase Positive Control	1 vial/50 µl	-80°C
700643	Thioglycerol Standard	1 vial/100 µl	-20°C
700029	96-Well Solid Plate (white)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3640
Email:	techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the capacity to measure fluorescence at an excitation wavelength between 380-390 nm and an emission wavelength between 510-520 nm
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Lipases perform essential roles in the digestion, transportation, and processing of dietary lipids. Elevated plasma triglyceride levels have been implicated as a risk factor for coronary heart disease (CHD). An inverse relationship exists between blood triglyceride and HDL levels. Low HDL levels are often associated with high triglyceride levels in both men and women, and the combination of low HDL and high triglyceride levels is related to an increase risk of developing CHD.[1] Plasma triglyceride levels are regulated by both synthesis and degradation of very low density lipoprotein (VLDL) and chylomicron particles. The clearance of triglyceride-rich lipoproteins from the circulation is controlled by the actions of lipoprotein lipase (LPL) and hepatic lipase (HL) and by the interlipoprotein exchange of triglyceride by cholesteryl ester transfer protein.[2] LPL is the predominant triglyceride lipase and is responsible for hydrolyzing triglycerides in chylomicrons and VLDL, whereas HL is both a phospholipase and a triglyceride lipase and plays an important role in HDL metabolism and in the conversion of VLDL to LDL.[3] LPL is mainly synthesized by adipocytes, skeletal muscle cells, and cardiac muscle cells, whereas HL is synthesized by the liver. The third well-characterized lipase from the triglyceride lipase family is endothelial lipase (EL). Endothelial lipase is synthesized mainly by vascular endothelial cells and to a lesser extent by macrophages and smooth muscle cells.[4] In contrast to LPL and HL, EL is primarily a phospholipase A_1 and hydrolyzes HDL-phospholipids at the sn-1 position. The EL level in plasma has been associated with HDL-C concentration and inflammation, which are important in the initiation and development of atherosclerosis.[5,6]

Free fatty acids stored as triglycerides in adipose tissue can be rapidly mobilized by the hydrolytic action of the three main lipases of the adipocyte: hormone-sensitive lipase (HSL), monoacylglyceride lipase (MAGL), and the newly discovered adipose triglyceride lipase (ATGL).[7-10] ATGL initiates lipolysis by specifically removing the first fatty acid from triglyceride to produce diacylglycerol, which is then hydrolyzed by HSL to generate an additional fatty acid and monoacylglycerol. Monoacylglycerol is converted to fatty acid and glycerol by MAGL in the final step of lipolysis. The fatty acids released are then used by other tissues during times of energy deprivation. The manipulation of lipolysis has therapeutic potential in the metabolic disorders frequently associated with obesity.[11]

The most widely used pharmacological therapies for obesity and weight management are based on the inhibition of gastrointestinal lipases, resulting in reduced dietary lipid absorption. Colipase-dependent pancreatic lipase and carboxy ester lipase are believed to be the major gastrointestinal enzymes involved in catalysis of lipid ester bonds.[12,13] Orlistat inhibits gastric, pancreatic, and carboxy ester lipases, preventing the hydrolysis of triglycerides to free fatty acids and monoglycerides, and as such is widely used to treat obesity.[14]

About This Assay

Cayman's Lipase Activity Assay provides a fluorescence-based method for detecting lipase activity in plasma, serum, tissue homogenates, and cell culture samples. In the assay, lipase hydrolyzes arachidonoyl-1-thioglycerol to arachidonic acid and thioglycerol. Thioglycerol reacts with the thiol fluorometric detector to yield a highly fluorescent product which can be analyzed with an excitation wavelength of 380-390 nm and an emission wavelength of 510-520 nm.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Sodium Phosphate Assay Buffer - (Item No. 700003)**

   This vial contains 10 ml of 250 mM Sodium Phosphate (pH 7.2). Mix the contents of the vial with 40 ml of HPLC-grade water. This final 1X Assay Buffer (50 mM Sodium Phosphate, pH 7.2) is used in the assay. The 1X buffer is stable for three months at 4°C.

2. **Fluorometric Thiol Detector - (Item No. 700006)**

   This vial contains 300 µl of Thiol Detector. Mix 120 µl of Thiol Detector with 480 µl of 1X Assay Buffer. This is enough detector to assay 50 wells. Prepare additional detector as needed. The diluted Thiol Detector is stable for four hours at room temperature. Store undiluted detector at -20°C.

3. **Lipase Substrate - (Item No. 700641)**

   This vial contains 1.2 ml of 500 µM arachidonoyl-1-thioglycerol in ethanol. It is ready to use in the assay. A 10 µl aliquot yields 25 µM substrate in the assay. When not using the substrate, store at -80°C.

4. **Lipase Positive Control - (Item No. 700642)**

   This vial contains 50 µl of bovine milk lipoprotein lipase. Mix 10 µl of the Lipase Positive Control with 140 µl of 1X Assay Buffer and put the vial on ice. The diluted enzyme is stable for two hours at 4°C. Store undiluted lipoprotein lipase at -80°C.

5. **Thioglycerol Standard - (Item No. 700643)**

   This vial contains 100 µl of 10 mM 1-thioglycerol. It is ready to use to prepare the standard curve (see page 12).

Sample Preparation

Cell Lysate

1. Collect cells (~1 x 10^6) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Sonicate cell pellet in 0.5-1 ml of cold 1X Assay Buffer or see **Interferences** (page 19) section for additional choices and vortex.
3. Centrifuge at 10,000 x g for 10 minutes at 4°C. Transfer the supernatant to a clean tube and store on ice.
4. If not assaying the same day, freeze at -80°C. The sample will be stable for one month while stored at -80°C.
5. Dilute the sample 1:2-1:4 with 1X Assay Buffer before assaying.

Tissue Homogenate

1. Prior to dissection, rinse tissue with a PBS (phosphate buffered saline solution, pH 7.4) to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of cold buffer (*i.e.*, 1X PBS, containing protease inhibitors of choice; see **Interferences** on page 19) per gram weight of tissue.
3. Centrifuge at 10,000 x g for 10 minutes at 4°C. Remove the supernatant and store on ice.
4. If not assaying the same day, freeze at -80°C. The sample will be stable for one month while stored at -80°C.
5. Dilute the sample 1:10-1:50 with 1X Assay Buffer before assaying.

Plasma

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 25°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer.
3. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
4. Dilute plasma 1:10-1:20 with 1X Assay Buffer before assaying.

Serum

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 25°C. Pipette off the top yellow serum layer without disturbing the white buffy layer.
4. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
5. Dilute serum 1:10-1:20 with 1X Assay Buffer before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a thioglycerol standard curve and lipase positive control in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate and in the presence and absence of lipase substrate. A typical layout of standards, positive control, samples, and sample backgrounds to be measured in duplicate is given below (see Figure 1).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17
B	B	B	B1	B1	B5	B5	B9	B9	B13	B13	B17	B17
C	C	C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18
D	D	D	B2	B2	B6	B6	B10	B10	B14	B14	B18	B18
E	E	E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19
F	F	F	B3	B3	B7	B7	B11	B11	B15	B15	B19	B19
G	G	G	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20
H	+	+	B4	B4	B8	B8	B12	B12	B16	B16	B20	B20

A-G = Thioglycerol Standards
+ = Lipase Positive Control
S1-S20 = Sample Wells
B1-B20 = Sample Background Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps to achieve more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- All reagents except the Lipase Positive Control and samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples at least in duplicate (triplicate preferred).
- The assay is performed at 37°C.
- Monitor the fluorescence with an excitation wavelength of 380-390 nm and an emission wavelength of 510-520 nm.

Standard Preparation

Mix 10 µl of the Thioglycerol Standard with 1.24 ml of 1X Assay Buffer to yield a stock concentration of 80 µM. Add the amount of thioglycerol stock (80 µM) and 1X Assay Buffer to each well of a dilution plate (or tube) as described in Table 1. The diluted Standards are stable for four hours at room temperature.

Well (or tube)	80 µM Thioglycerol Stock (µl)	1X Assay Buffer (µl)	Final Thioglycerol Concentration (µM)
A	0	200	0
B	5	195	2
C	10	190	4
D	25	175	10
E	50	150	20
F	100	100	40
G	200	0	80

Table 1. Preparation of the Thioglycerol Standards

Performing the Assay

1. **Standard Wells -** add 170 µl of 1X Assay Buffer, 10 µl of Thiol Detector, 10 µl of Lipase Substrate, and 10 µl of Standard (wells or tubes, A-G) per well in the designated wells on the plate (see **Sample plate format**, Figure 1, page 10).
2. Cover and incubate the plate for 15 minutes at 37°C. Read the fluorescence using an excitation wavelength of 380-390 nm and an emission wavelength of 510-520 nm. Reading the standards prior to measuring sample activity allows an appropriate *gain* to be established for detecting the entire range of standards. This *gain* must be used when assaying the samples.
3. **Lipase Positive Control Wells -** add 170 µl of 1X Assay Buffer, 10 µl of Thiol Detector, and 10 µl of the Lipase Positive Control to at least two wells.
4. **Sample Wells -** add 170 µl of 1X Assay Buffer, 10 µl of Thiol Detector, and 10 µl of sample to at least two wells.
5. **Sample Background Wells -** add 180 µl of 1X Assay Buffer, 10 µl of Thiol Detector, and 10 µl of sample to at least two wells.
6. Initiate the reactions by quickly adding 10 µl of Lipase Substrate to the Lipase Positive Control and Sample Wells. DO NOT add the Lipase Substrate to the Sample Background Wells.
7. Read the fluorescence at 37°C every 30 seconds for 15 minutes using an excitation wavelength of 380-390 nm and an emission wavelength of 510-520 nm.

Well	1X Assay Buffer (µl)	Thiol Detector (µl)	Lipase Positive Control (µl)
Lipase Positive Control	170	10	10
Sample	170	10	10
Sample Background Wells	180	10	10

Table 2. Pipetting summary

ANALYSIS

Calculations

Plot the Standard Curve

1. Determine the average fluorescence of each standard. Subtract the fluorescence value of the standard A from itself and all other standards. This is the corrected fluorescence.
2. Plot the corrected fluorescence values (from step 1 above) of each standard as a function of the final concentration of thioglycerol from Table 1. See Figure 2, on page 17, for a typical standard curve.

Determine Lipase Activity

1. Determine the average fluorescence of each sample and sample background at each time point. Plot fluorescence as a function of time.
2. Determine the change in fluorescence (RFU) per minute by:
   a. Obtain the slope (rate) of the linear portion of the curve. An example of lipoprotein lipase positive control is shown in Figure 3, on page 18.

   or

   b. Select two points on the linear portion of the curve and determine the change in fluorescence during that time using the following equation:

$$\text{RFU/min} = \frac{\text{RFU (Time 2) - RFU (Time 1)}}{\text{Time 2 (min) - Time 1 (min)}}$$

3. Subtract the sample background rate (RFU/min) from that of the sample.
4. Calculate the lipase activity using the following equation. One unit is defined as the amount of enzyme that will cause the formation of 1 nmol of thioglycerol per minute at 37°C.

Lipase Activity (nmol/min/ml) =

$$\left[\frac{\text{RFU/min}}{\text{Slope from standard curve (RFU/}\mu\text{M)}}\right] \times \text{Sample dilution}$$

Sample Data:

The data shown here is an example of the data typically produced with this kit. Your results may vary, and therefore should not be directly compared to these samples.

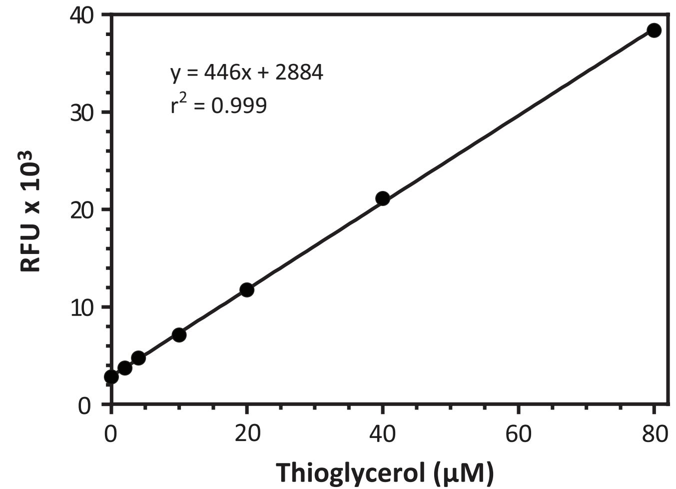

Figure 2. Thioglycerol standard curve

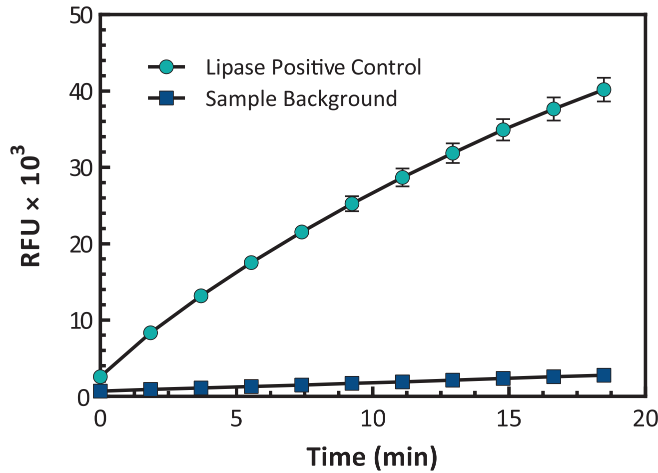

Figure 3. Lipoprotein Lipase Positive Control assayed with and without Lipase Substrate

Performance Characteristics

Sensitivity:

The limit of detection for the assay is 0.1 U/ml (±0.05 U/ml) lipase.

Precision:

When a series of 16 human plasma measurements were performed on the same day, the intra-assay coefficient of variation was 4.8%. When a series of human plasma measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 5.1%.

RESOURCES

Interferences

The following reagents were tested for interference in the assay:

	Reagent	Will Interfere (% interference)
Buffers	HEPES	No
	Phosphate	No
	1X Phosphate Buffered Saline	No
	Tris	No
Chelators	EDTA (1 mM)	No
	EGTA (1 mM)	No
Detergents	Polysorbate 20 (0.1%)	No
	Polysorbate 20 (1%)	Yes (10%)
	Triton X-100 (0.1%)	No
	Triton X-100 (1%)	Yes (65%)
Protease Inhibitors/ Enzymes	Antipain (10 µg/ml)	No
	Chymostatin (10 µg/ml)	No
	Leupeptin (10 µg/ml)	No
	Trypsin (10 µg/ml)	No
Solvents	Dimethylsulfoxide (5%)	No
	Ethanol (5%)	No
	Methanol (5%)	No
Others	BSA (0.1%)	No
	Cysteine	Yes
	Dithiothreitol (1 mM)	No
	Glutathione	Yes
	Glycerol (10%)	No
	NaCl (100 mM)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the well(s) B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence was detected above background in the sample wells	A. The lipase concentration is too low to detect B. The sample does not contain lipase, or the sample contains something that is interfering	A. Re-assay the sample using a lower dilution B. Check the **Interferences** section for possible interferences (see page 19)
Sample fluorescence was above highest point in standard curve	A. Lipase concentration was too high in the sample B. The sample was too concentrated	Dilute samples with 1X Assay Buffer and re-assay; *NOTE: Remember to account for the dilution factor when calculating lipase concentration*
The plate reader exhibited "MAX" values for the wells	The *gain* setting is too high	Reduce the *gain* and re-read

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A												
B												
C												
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F												
G												
H												

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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