Code No. 27183

Human HTGL Assay Kit - IBL

INTRODUCTION

HTGL (hepatic triacylglycerol lipase / hepatic triglyceride lipase / (HL) hepatic lipase), a lipolytic enzyme, is a secreted glycoprotein. HTGL plays a major role in lipoprotein metabolism as a lipolytic enzyme that hydrolyzes triglycerides (TGs) and phospholipids in chylomicron remnants, intermediate-density lipoproteins (IDLs), and high-density lipoproteins (HDLs). It has been reported that the persons with HTGL deficiency present with hypercholesterolemia or hypertriglyceridemia and accumulate β-very-low-density lipoproteins (VLDLs), chylomicron remnants, IDLs, TG-rich low-density lipoproteins (LDLs) and HDLs.
HTGL is synthesized by hepatocytes and bound to heparin sulfate proteoglycans at the surface of liver sinusoidal capillaries. Measurements of HTGL in plasma have been routinely carried out after intravenous injection of heparin (**postheparin plasma**) as well as lipoprotein lipase (LPL). Because of the highbinding affinity of heparin for the lipases, the enzyme bound to endothelial surfaces is rapidly released by heparin from capillary beds into plasma.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human HTGL.

MEASUREMENT RANGE

0.47 - 30 ng/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination of human HTGL in postheparin plasma collected with EDTA and cell culture supernatant.
The guideline of dilution rate for postheparin plasma collected with EDTA is around 20 times.

KIT COMPONENT

1	Precoated plate : Anti-Human HTGL (31A1) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human HTGL (26A1) Mouse IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant human HTGL	0.5mL x 2
4	EIA buffer	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc. *	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

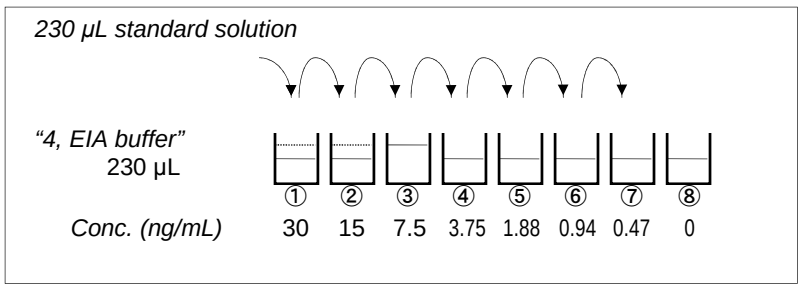
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 60 ng/mL humn HTGL standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	30 ng/mL
Tube-2	15 ng/mL
Tube-3	7.5 ng/mL
Tube-4	3.75 ng/mL
Tube-5	1.88 ng/mL
Tube-6	0.94 ng/mL
Tube-7	0.47 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 30 ng/mL and 0.47 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
Test postheparin plasma samples have to be diluted around 20-fold with "4, EIA buffer" in the kit necessarily.
Dilute cell culture supernatant samples suitably with "4, EIA buffer" as necessary.
The pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation overnight at 4 °C with plate lid				
4 times (wash buffer more than 350 µL) *				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL) *				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate overnight at 4 °C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid. *
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid. *
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", suitably. Refer to 2-5).
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer in this kit for washing the precoated plate.
Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.

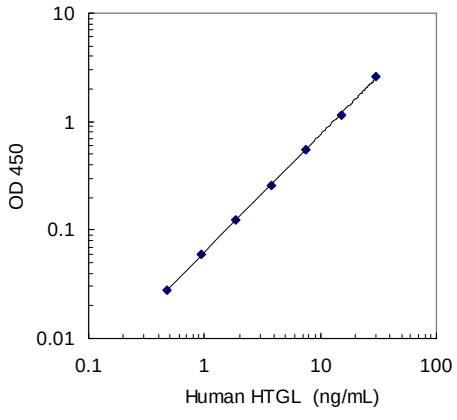
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

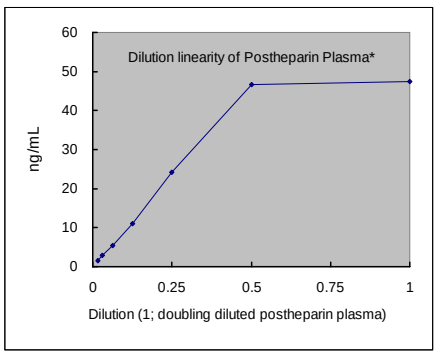
Conc. (ng/mL)	Absorbance (450nm)
30	2.600
15	1.165
7.5	0.554
3.75	0.266
1.88	0.130
0.94	0.067
0.47	0.035
0 (Test Sample Blank)	0.007

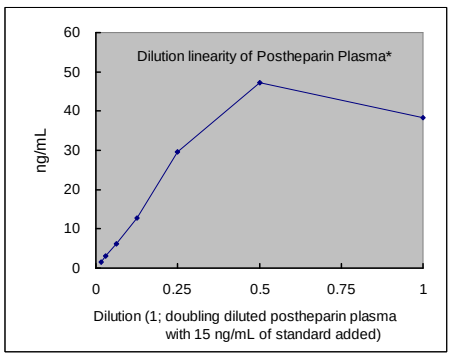


* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

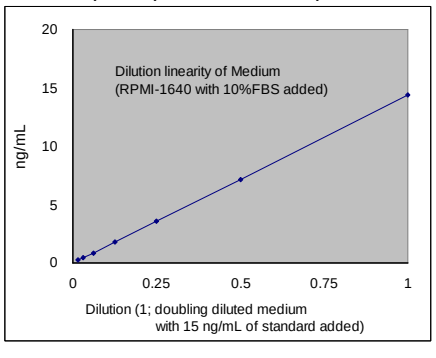
PERFORMANCE CHARACTERISTICS

1. Dilution linearity





*Postheparin plasma: EDTA-plasma collected after intravenous injection of heparin.



2. Added Recovery Assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measured Value (ng/mL)	%
Postheparin Plasma (EDTA) (x8)	3.75	21.39	24.47	114.4
	1.88	19.52	19.58	100.3
	0.94	18.58	19.85	106.8
Medium with 10% FBS (x4)	15	15	14.86	99.1
	7.5	7.5	7.27	96.9
	3.75	3.75	3.68	98.1

3. Intra - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
13.75	0.71	5.1	24
3.37	0.17	5.0	24
1.19	0.07	5.9	24

4. Inter - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
14.09	0.96	6.8	5
3.36	0.15	4.5	5
1.16	0.08	6.9	5

5. Specificity

Substance	Cross-Reactivity
Human HTGL	100 %
Human LPL	< 0.1 %
Human EL	< 0.1 %

6. Sensitivity

0.08 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

REFERENCE

1. Miyashita K, Kobayashi J, Imamura S, Kinoshita N, Stanhope KL, Havel PJ, Nakajima K, Machida T, Sumino H, Nara M, Murakami M. A new enzyme-linked immunosorbent assay system for human hepatic triglyceride lipase. Clin Chim Acta. 2013 Sep 23;424:201-6.
2. Imamura S, Kobayashi J, Sakasegawa S, Nohara A, Nakajima K, Kawashiri M, Inazu A, Yamagishi M, Koizumi J, Mabuchi H. A novel method for measuring human hepatic lipase activity in postheparin plasma. J Lipid Res. 2007 Feb;48(2):453-7.

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Made in Japan.