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Reagent for quantification of Lipoprotein lipase (LPL) in plasma or serum

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MARKIT-M LPL

■Introduction

Lipoprotein lipase (LPL) is a key enzym e for the m etabolism of trig lyceride (TG)-rich lipoproteins, and functionally it hydrolyzes the TGs in chylom icrons and very low-density lipoprotein (VLDL) in the blood . It is well known that continual hyperlipoproteinemia is a high ri sk factor for arteriosclerosis and myocardial infarction. In order to investigate functional abnorm alities of LPL in genetic d isorders and in secondary hyperlipoproteinem ia, m easurement of LPL is very im portant, and it is also very informative for medical treatm ent of hyperlipoproteinemia. It is not easy to precisely as sess enzyme activity of LPL due to interf erence by hepatic TG lip ase (HTGL) and other lipases present in hum an blood. In cooperation with Dr . Y. Ikeda and Dr. A. T akagi (National Cardiovascular Center Research Instit ute, Osaka, Japan), we have developed MARKIT -M LPL kit, wh ich is a direct sandwich enzym e-linked immunosorbent assay (ELISA) for the quantif ication of LPL in hum an plasm a us ing two distinct anti-hum an LPL m onoclonal antibodies. MARKIT -M LPL kit gives excellent accuracy and specificity , good reproducibility and rapidity in the quantification of the LPL mass in hum an bl ood. The LPL m ass concentrations measured by this kit co rrelate well with LPL mass values d etermined by the one-step sandwich-EIA (MARKIT-F LPL EIA kit) previ ously established by us. In addition, the LPL mass values correlate well with the LPL enzyme activity.

■Contents of MARKIT-M LPL

Each kit (96 tests) contains the following reagents.
Standard 0 (lyophilized): 1 vial (for 0.5 mL).
Standard 25 (lyophilized): 1 vial (for 0.5 mL) contains: T-LPL* 12.5 ng.
Standard 50 (lyophilized): 1 vial (for 0.5 mL) contains: T-LPL* 25 ng.
Standard100 (lyophilized): 1 vial (for 0.5 mL) contains: T-LPL* 50 ng.
Standard 200 (lyophilized): 1 vial (for 0.5 mL) contains: T-LPL* 100 ng.
Standard 300 (lyophilized): 1 vial (for 0.5 mL) contains: T-LPL* 150 ng.
Stabilizer solution (bottle No. 1): 1 bottle (14 mL).
Wash buffer concentrate (bottle No. 2): 1 bottle (90 mL).

LPL antibody-enzyme conjugate (bottle No. 3): 1 bottle (14 mL).
HRP-labeled anti-human LPL monoclonal antibody (mouse)
LPL antibody-coated wells: 1 plate (96 wells).
Anti-human LPL monoclonal antibody (mouse)
Substrate tablet: 3. One tablet contains : o-phenylenediamine dihydrochloride (OPD) (13 mg).
Substrate diluent buffer (bottle No. 4): 3 bottles (15 mL each). One bottle contains: hydrogen peroxide (15 μL).
Stop solution (bottle No. 5): 1 bottle (15 mL).
Microplate for dilution: 1 plate (96 wells).
Graph paper: 1 sheet.

*T-LPL: T-LPL is abundantly produced and secreted by THP-1, which is a human myelogenous leukemia cell line. T-LPL is purified from the cultured medium of THP-1 and is immunologically homologous with LPL in human postheparin plasma (PHP), but it has no enzyme activity. The content of immunoreactive LPL in the standard reagent was determined immunologically by using purified human LPL as the standard substance.

■Application

Quantification of LPL in human plasma or serum.

■Principle

Two-step direct sandwich enzyme-linked immunosorbent assay (ELISA) using two distinct mouse anti-human LPL monoclonal antibodies.

■Assay method

1. Instruments and materials required

Pipettes with disposable tips 25, 500 μL, Multichannel pipette 100, 300 μL, Volumetric cylinder 1000 mL, ELISA washer, Microplate reader equipped with 492 nm (as the main wave length) and 620 nm (as the reference wave length).

2. Preparation of sample

After the subjects have fasted overnight, blood samples should be collected early in the morning.

(1) Pre HP

When collecting plasma, obtain the blood in the collection tube containing

anticoagulant (disodium EDTA or heparin).

(2) PHP

Collect blood 10 m in after heparin (sodium salt) I.V. injection at 10-30 units/kg of body weight, then pour the blood into the blood collection tube containing an anticoagulant and mix well. Within 8 hrs, centrifuge the blood collection tube (1500xg, 10 min) and collect the supernatant (PHP) as the sample for determination of LPL. In case heparin is administered at 50 units/kg, collection of blood should be done 15 min later.

(3) Keep samples in ice water until assay. In case of storage of samples, keep them frozen under –20°C.

3. Preparation of reagents

(1) Standard solutions

Accurately add 0.5 mL of purified water to the standard reagent vials. Stand for 15 min and then shake the vials gently to dissolve the contents thoroughly. (Standard solutions are stable for at least 1 month at 2-10°C or at least 3 months at –20 °C. No influence on the stability was observed after 10 cycles of freezing-thawing.)

(2) Wash buffer

Put the whole volume of the wash buffer concentrate (bottle No. 2) into a 1000-mL volumetric cylinder and dilute with purified water to 900 mL. Use this as a wash buffer. (Wash buffer is stable for at least 1 week at 2-10°C.)

(3) Substrate solution

Put one substrate tablet (OPD) into one bottle of substrate diluent buffer (bottle No. 4), mix gently and use it as the substrate solution. Prepare the substrate solution just before use, and keep it shielded from light.

4. Procedure

See the following methods and figure. It is preferable to determine standard solutions in duplicate.

(1) 25 μL of sample or standard solution is mixed with 100 μL of stabilizer solution (bottle No. 1) predispensed into the wells of a microplate for dilution.

(2) The mixture of sample or standard solution and stabilizer solution (100 μL) is dispensed into LPL antibody-coated wells, and then the plate is agitated for 30 sec.

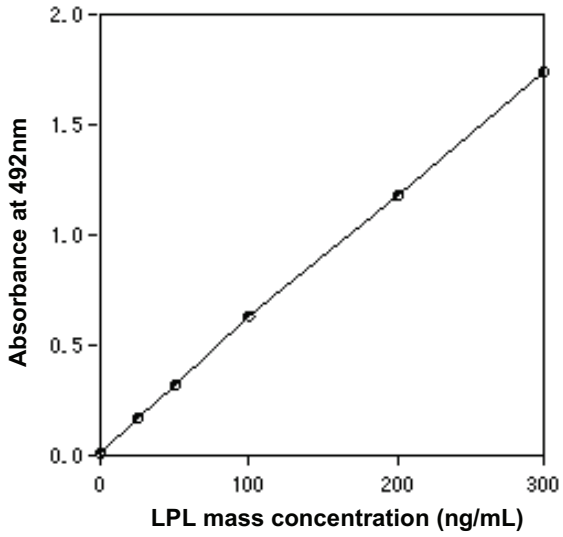
(3) LPL antibody-coated wells are incubated for 30 min at 15-25°C.

(4) LPL antibody-coated wells are washed three times with wash buffer (300 μL/well) to remove unbound LPL molecules.

(5) LPL antibo dy-enzyme conjugate (bottle No. 3) (100 μL) is added to LPL antibody-coated wells, and the microplate is agitated for 30 sec.
(6) The microplate is incubated for 30 min at 15-25°C.
(7) The m icroplate is washed three tim es with wash buf fer (300 μL/well) to remove unreacted LPL antibody-enzyme conjugate.
(8) Substrate so lution (100 μL) is add ed to each well to as say the HRP enzyme activity.
(9) The microplate is shielded from light and incubated for 30 min at 15-25°C.
(10) The HRP enzym e reaction is term inated by addition of 100 μL of stop solution (bottle No. 5) to each well, and then the plate is agitated for 30 sec.
(11) The absorbance of each well at 492 nm (main wave length) and 6 20 n m (reference wave length) is measured using a microplate reader within 3 hrs.
(12) The concentration of the LPL mass in each sample is calculated by reference to the standard curve ob tained from the six points of the standard solutions and expressed as ng of LPL protein/mL of plasma.

5. Preparation of a standard curve and reading of LPL concentration in samples

(1) The graph paper for preparation of a standard curve included in the kit plots the absorbance on the ordin ate and the concen tration of each sta ndard solution on the abscissa. Plot the absorbance o btained by u sing each standard solu tion of the corresponding LPL concentration and draw the best-fit smooth curve.
(2) Read the LP L concentration corresponding to the absorban ce of the sam ple by using the standard curve. The obtai ned value directly indicates the LPL concentration (ng/mL).
(3) Indicate as “below 3.6 ng/mL” when the obtained value is below the lower limit of detection (3.6 ng/mL). For quantif ication of high-concentration sam ples (more than 300 ng/m L), dilute the sam ples properly with standard solution 0. Then perf orm the whole procedure and m ultiply the obtained value by the dilution factor for correcti on. (Caution: Never dilute the samples with purified water.)



Typical standard calibration curve

■_Referenced data of LPL concentration

Reference data of LPL concentration (ng/mL) in PHP using 30 units/kg of heparin

Pre HP	LPL abnormal		LPL normal	Reference
	< 30.2 ng/mL		≧ 30.2 ng/mL	Ref. 4)
PHP	LPL homozygous deficiency	LPL heterozygous deficiency	Normal Subjects	Reference
	<50 ng/mL	50-140 ng/mL	140-353 ng/mL	Ref. 5)

■Instruction in procedure

1. Storage of samples

Seal tubes containing sam ples with a rubber stopper , etc, if they will not be assayed within 24 hrs, and preserve them at –20°C until use.

2. Freezing-thawing of samples

No influence on the determination was observed after 5 cycles of freezing-thawing.

■Interference of co-existing substances and drugs

1. Maximum permissible level of interfering substances

Hemoglobin : 470 mg/dL, bilirubin : 25.9 mg/dL, trigly ceride : 5, 000 m g/dL, creatinine : 487.4 mg/dL, uric acid : 50.8 mg/dL

2. Permissible level of interfering drugs

To evaluate possible interference by the following typical anti-hyperlipemia drugs and anti-diabetic drugs, they were added to PHP at various concentrations (these are 1.25 times higher than the maximum plasma concentration of each drug). Practically no influences on the result were found.

(1) Anti-hyperlipemia drugs:

Pravastatin-Na (18 ng/mL), probucol (20.5 μg/mL), bezafibrate (4.48 μg/mL), clinofibrate (25.6 μg/mL), clofibrate (76.8 μg/mL), elastase (0.72 ng/mL)

(2) Anti-diabetic drug:

Tolazamide (8.3 μg/mL), acetohexamide (64 μg/mL), gliclazide (13.3 μg/mL), glibenclamide (0.26 μg/mL), tolbutamide (50 μg/mL)

■Performance

1. Sensitivity

The measured absorbance "A" of standard solutions 0, 25, 50, 100, 200 and 300 should be as follows:

(1) A(300) minus A(0) is more than 0.8.

(2) A(0)<A(25)<A(50) <A(100)<A(200)<A(300)

(3) Absorbance of standard solution 12.5 prepared using standard solution 0 and 25: A(12.5) should be larger than A(0) and smaller than A(25).

2. Specificity

Control plasma (LPL, 100-240 ng/mL) should show a value within 85-115% of its known concentration with this kit.

3. Reproducibility

When two distinct samples (LPL, 100-240 ng/mL) are determined 10 times each simultaneously, the coefficient of variation in their absorbance should be less than 5%.

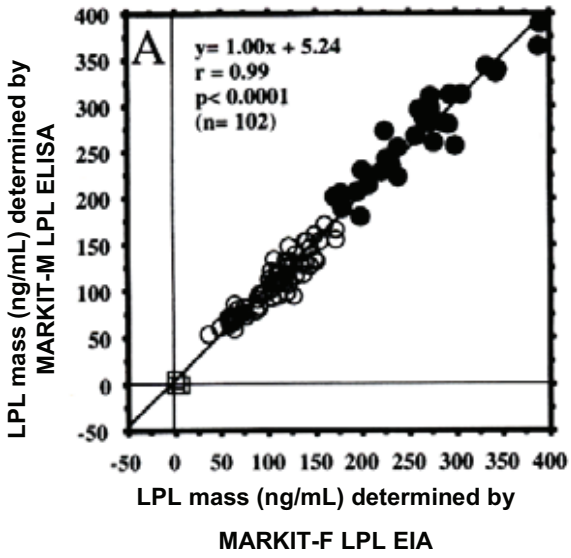
■Range of standard curve

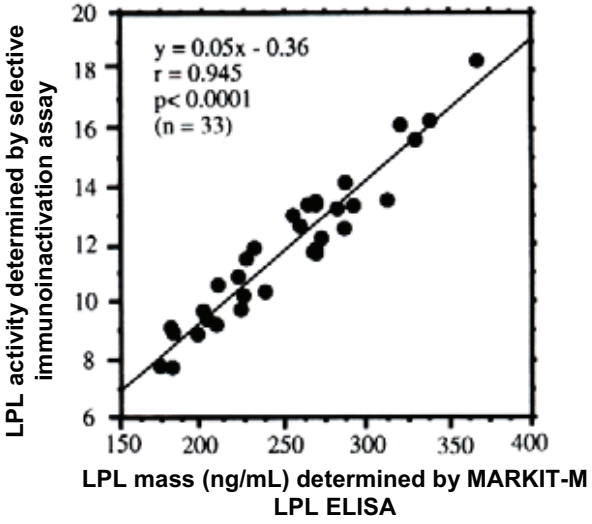
LPL 0-300 ng/mL

■Correlation between MARKIT-M LPL and MARKIT-F LPL and LPL enzyme activity

The regression curve between the LPL mass measured by MARKIT-F LPL (X) and by MARKIT-M LPL (Y) showed good correlation as follows: Y=1.00X+5.24, r=0.99 (n=102). On the other hand, the correlation between the LPL mass (X) measured by MARKIT-M LPL and the LPL activity (Y) determined by selective immunoinactivation

assay was also good, r=0.945 (n=33).





■Precautions for use or handling

1. General Precautions

(1) Be sure to use LPL antibody-enzyme conjugate and LPL antibody-coated wells of the same lot.

(2) The sample assay is recommended to be performed in duplicate until the technician becomes experienced.

(3) All reagents should be added in the exact order stated in the procedures. Samples and standard solutions should be treated under the same conditions.

2. Avoiding hazards to the user

(1) Viruses

The human plasma used as the standard in this kit is negative for HBs antigen, HIV antibody and HCV antibody.

(2) Pipetting

Never use your mouth to pipette the reagents or samples at any time. Never fail to use a pipette with disposable tips.

3. Handling of waste

Inactivate viruses in samples, reagents and used apparatuses when the test is completed by the following methods.

(1) Autoclave (132°C, 1 hr).

(2) Submerge in 1-5% sodium hypochlorite solution at room temperature for 2 hrs.

(3) Submerge in 1 mol/L sodium hydroxide solution at room temperature for 1 hr.

(4) Submerge in 3% sodium dodecyl sulfate solution at 100°C for 5 min.

■Storage method and expiry period

Storage : Store at 2-10°C, protected from light. Avoid freezing.

Expiry period: The expiration date is printed on the outer box.

■Package units

MARKIT-M LPL: 1 kit (96 tests)

■References

Papers related to the establishment of one-step LPL EIA (MARKIT-F LPL) and LPL ELISA (MARKIT-M LPL)

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Others

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