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| Please read carefully this document prior to use |
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Code Number: 27264

LPL / HTGL Activity Control Plus Kit- IBL

【General Caution】

1. This product is used for research and it shall not be used for any other purpose.
2. No guarantee is applied for any other method of use other than this attached document.
3. Carefully read an attached document and/or any handling procedure related to an equipment intended to use for the test before use.

【Contents】

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| ① R1A Reagent (For HTGL activity, Freeze-dry) | 5mL x 2 |
| ② R1B Reagent (For HTGL+LPL activity, Freeze-dry) | 5mL x 2 |
| ③ R1 Solvent | 5mL x 4 |
| ④ R2 Color Reagent | 5mL x 2 |
| ⑤ Calibrator (Common to both HTGL and HTGL+LPL, Freeze-dry) | 0.5mL x 2 |
| ⑥ LPL/HTGL Activity Control H(Freeze-dry) | 0.3mL x 1 |
| ⑦ LPL/HTGL Activity Control L(Freeze-dry) | 0.3mL x 1 |

【Intended Use】

This kit can measure the activities of lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) in postheparin plasma. LPL plays a central role in lipoprotein metabolism by catalyzing hydrolysis of triglycerides (TGs) in chylomicrons and VLDL particles. Patients with LPL deficiency present with marked hypertriglyceridemia with accumulation of chylomicrons.

HTGL is synthesized by hepatocytes and bound to heparin sulfate proteoglycans at the surface of liver sinusoidal capillaries which hydrolyzes TGs and phospholipids in chylomicron remnants, intermediate density lipoproteins, and HDLs. Patients with HTGL deficiency present with hypercholesterolemia and hypertriglyceridemia and accumulation of β -VLDLs, chylomicron remnants, IDLs and TG-rich LDLs and HDLs.

【Principal for Measurement】

LPL and HTGL act on a natural substrate, diglyceride to liberate monoglyceride. This is hydrolyzed by monoglyceride lipase (absolutely high specificity for monoglyceride) into glycerol and free fatty acid. Glycerol kinase acts on glycerol to glycerol-3-phosphate, which is in turn acts on by glycerol-3-phosphate oxidase to generate hydrogen peroxide.

Peroxidase converts the hydrogen peroxide, 4-Aminoantipyrine and TOOS(N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine) into quinoneimine dye. The rate of formation of the dye, measured as an increase in absorbance at 550nm, is proportional to the lipase.

LPL requires apolipoprotein CII (apoCII) for the lipase activity,

in contrast HTGL. The both lipase activities with apoCII and without apoCII are measured with 2 channel of autoanalyzer.

LPL and HTGL activities can be calculated the difference between with apoCII and without apoCII.

【Caution for Operation】

1. Feature of test samples and method of collecting the samples.

- (1) EDTA-plasma(postheparin) should be used as a test sample. In that case 30 or 50 unit of heparin (heparin sodium injection liquid produced under the Japanese Pharmacopoeia) per 1kg weight is injected by the intravenous injection, the peak concentration of the both lipases in blood is indicated 10 to 15 minutes after the injection. Dosage of heparin and timing of collection should be consistent in the blood samples.
- (2) Collected samples after centrifugation should be stored in a freezer at -20°C or lower if the sample is not measured on the same day of collection. The freeze-thawed sample can be used only once. Stored frozen samples are used after thawed at room temperature.
- (3) Please be careful that the sample is not bubbled when it is dispensed.

2. Interfering Substances

- (1) Free bilirubin does not affect on the value of measurement up to 20 mg/dL.
- (2) Conjugated bilirubin does not affect on the value of measurement up to 20 mg/dL.
- (3) Hemoglobin does not affect on the value of measurement up to 500 mg/dL.
- (4) Chyle does not affect on the value of measurement up to 1,400 FTU.

【Method】

1. Preparation

- (1) R1A : Reconstitute R1A Reagent vial with the R1 solvent by pouring the content of the R1 solvent into R1A vial. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use.
- (2) R1B : Reconstitute R1B Reagent vial with the R1 solvent by pouring the content of the R1 solvent into the R1B vial. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use.
- (3) R2 Color Reagent: Ready to use.
- (4) Calibrator: Reconstitute with 500 μ L of purified water. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use. The concentration of the calibrator is described on the label of each vial.
- (5) Activity control H and L: Reconstitute with 300 μ L of

purified water. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use. The concentration of each control is listed on the label of each vial.

- (6) Separately, a physiological saline solution is required as a reagent blank.

2. Operational Procedure

(In the case for autoanalyzer.)

- (1) Two channels (R1A, R1B) are necessary. One channel R1A is for HTGL activity. Another channel R1B is for HTGL+LPL activity. Set R1A, R1B and R2 Color reagent each on autoanalyzer.
- (2) Input the value of R1A and R1B of Calibrator. The value of each activity is listed on a label of vial.
- (3) Parameters
R1A : 160 μ L
R1B : 160 μ L
Sample; 3 μ L
Incubation for 5 minutes at 37°C
R2 Color Reagent: 80 μ L
Measuring wave length: 550nm
Sub-wave length: 660nm
Measure the increasing velocity after 3min after R2 Color Reagent addition.
- (4) The value of LPL activity is obtained by subtracting the value of R1A (HTGL activity) from the value of R1B (HTGL+LPL activity).

【Performance】

1. Performance

(1) Sensitivity

With R1A, the increase of Optical Density (OD) of reagent blank should be less than 5mAbs, and the difference between calibrator and reagent blank should be over than 20mAbs.

With R1B, the increase of Optical Density (OD) of reagent blank should be less than 5mAbs, and the difference between calibrator and reagent blank should be over than 28mAbs.

(2) Precision

The measurement value of control sample that has already been determined its concentration is 80 ~120% of determined value.

(3) Intra-Assay

Obtained CV is less than 15% if same control sample is measured 5 times.

2. Measurement Range

HTGL activity: 135 ~ 431 U/L

LPL activity: 30 ~ 153 U/L

(Used Tokyo Boeki Biolis24iP type automated analyzer)

Please note: Internal testing method is applied.

【Precaution for Intended Use and/or Handling】

1. Caution for Handling (Hazard Prevention)

- (1) Any sample should be carefully handled as it might have a risk of infection such as HIV, HBV and HCV.
- (2) All reagents contain sodium azide as a preservative and it may cause irritation on skin. Immediately wash

it out with a plenty of water if any reagent is mistakenly contacted with eyes, mouth and skin and seek a medical consultant if necessary.

- (3) Some reagents contained in this product contain animal origin substances. Immediately wash it out with a plenty of water if any reagent is mistakenly contacted with eyes, mouth and skin and seek a medical consultant if necessary.

2. Caution for Use

- (1) Do not use any reagent has already been expired as the reliability of any measured values cannot be assured.
- (2) Do not use frozen reagents.
- (3) Do not use any reagent combination with other lots.
- (4) The reagents should be immediately used after reconstitution without freezing and thawing.

3. Caution for Disposal of Reagents

- (1) Each reagent of this kit contains sodium azide as a preservative. Wash it out with a plenty of water if any reagent of this kit is disposed as sodium azide may react with copper and it may cause explosion due to the chemical reaction.
- (2) Any equipment or solution used for samples and tests should be incinerated or sterilized by sodium hypochlorite (over 1 hour with an effective chloride concentration: 1,000ppm), glutaraldehyde (over 1 hour with 2% concentration) or autoclave treatment (over 20 minutes at 121°C).
- (3) Any reagent or equipment shall be disposed complying with any disposal treatment and/or cleaning relevant laws or regulations such as water quality pollution control Act.

【Storage Control and Shelf Life】

This product shall be stored at 2 ~ 10°C.

Shelf life is labeled on outer package of this product.*

【Related Products Available with an Additional Charge】

| Product Code | Product Name | Package Size |
|--------------|---------------------------------|--------------|
| 27185 | LPL/HTGL Activity Assay Kit-IBL | 1 Kit |

【Manufacturer】



Customer Support

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