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Pre-β1-HDL ELISA
Product No. 289194

Storage: 2–10°C

For Research Use Only!

INTENDED USE

Pre-β1-HDL ELISA is a quantitative enzyme-linked immunosorbent assay (ELISA) kit for Pre-β1-HDL in human plasma. The product is intended for research use only.

EXPLANATION OF THE TEST

High density lipoproteins (HDL) are defined as lipoproteins in the density range of 1.0–1.2 kg/l. HDL particles are heterogeneous in size, apolipoprotein composition, and function.

Pre-β1-HDL is a specific HDL subfraction that migrates with pre-β mobility on agarose gel electrophoresis. The main components of pre-β1-HDL are apolipoprotein A1 (apoA1) and phospholipids. Pre-β1-HDL is an acceptor of cellular cholesterol and is critical for reverse cholesterol transport.

In normal plasma, lecithin:cholesterol acyltransferase (LCAT) converts pre-β1-HDL to α-migrating HDL, which transports esterified cellular cholesterol to the liver for further processing.

Plasma pre-β1-HDL levels have been reported to be increased in patients with coronary artery disease (CAD) and dyslipidemia. However, the mechanism responsible for elevation of the pre-β1-HDL level has not been clarified.

It has been demonstrated that the pre-β1-HDL concentration is elevated in type 2 diabetic patients and that a high pre-β1-HDL concentration is a predictor of carotid atherosclerosis. Many prospective studies have reported positive correlations between the severity of carotid atherosclerosis and cardiovascular risks in general populations and diabetic patients. Accelerated atherosclerosis in diabetic patients may be explained by insulin resistance, chronic inflammation, hyperglycemia, and dyslipidemia. LCAT-dependent conversion of pre-β1-HDL into α-migrating HDL is severely delayed in hemodialysis patients.

PRINCIPLE OF THE METHOD

Test wells are coated with anti-pre-β1-HDL monoclonal antibody. pre-β1-HDL in the sample is captured by the antibody in the 1st incubation. After the 1st incubation and washing to remove all of the unbound material, Enzyme-labeled anti-apolipoprotein A1 polyclonal antibody is added. After the 2nd incubation and subsequent washing, substrate solution is added. Next, stop reagent is added. The intensity of color that develops is read by a microplate reader. The absorbance is proportional to the concentration of pre-β1-HDL in the sample.

REAGENTS

- MTP** anti-pre-β1-HDL mAb coated microtiter plate (96 wells), 1 plate
- STAB** Stabilization buffer, 20 ml
- DILB** Dilution buffer, 100 ml
- WASH1** Wash buffer concentrate-1, 50 ml
- CON** Enzyme-labeled anti-apolipoprotein A1 pAb, 7 ml
- WASH2** Wash buffer concentrate-2, 50 ml
- SUB** Substrate (lyophilized), 6 ml, 2 vials
- SUBB** Substrate buffer, 15 ml
- STOP** Stop Reagent, 15 ml
- STD** Calibrator (lyophilized), 2 vials

MATERIAL REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measurement at 492 nm
- 8-channel pipet covering 50–200µL
- 1-channel pipet covering 20–1000µL
- Deionized or distilled water
- Plastic test tube
- Volumetric flask of cylinder (1000 mL)
- Absorbent paper towels
- Micro-plate shaker with horizontal circular movement, if available
- Plate washer, automated or manual, if available

REAGENT PREPARATION AND STORAGE

Reagents before preparation are stable for 2 years at 2–10°C.

Allow all reagents to equilibrate to room temperature (20–25°C) prior to use.

WASH1 Dilute the wash buffer concentrate-1 with 450 ml of distilled water. The diluted buffer solution is stable for 2 weeks at 2–10°C.

WASH2 Dilute the wash buffer concentrate-2 with 450 ml of distilled water. The diluted buffer solution is stable for 2 weeks at 2–10°C.

SUB Just prior to use, reconstitute the substrate (lyophilized) by adding 6 ml of substrate buffer **SUBB** to the vial. Since the substrate is light sensitive, it should not be exposed to excessive light. The substrate solution should be used within 1 hour after reconstitution.

STD Just prior to use, reconstitute the standard (lyophilized) by adding an appropriate volume^(*) of the dilution buffer **DILB** to the calibrator vial in order to prepare a concentration of 100 ng/ml. The content of pre-β1-HDL is indicated on the label. The following serial dilution series (C0–C7) for the calibration curve should be prepared.

^(*) in a case of calibrator lot 001PEX, add 1,420 µl of the dilution buffer.

Concentration (ng/ml)	C7	C6	C5	C4	C3	C2	C1	C0
100	50	25	12.5	6.25	3.13	1.56	0	
Calibrator [µl]	150	150	150	150	150	150	150	0
Dilution buffer [µl]	0	150	150	150	150	150	150	150

PREPARATION OF SAMPLES

1. Dilute the plasma^(*) to 21-folds with the stabilization buffer. (For example: Add 10 µl of plasma to 200 µl of stabilization buffer.)

^(*) Please refer to the PROCEDURE NOTES.

2. Just prior to assay, a 21-folds diluted sample should be diluted additionally to 101-folds with the dilution buffer (c).

Please note that the dilution rate from the original plasma would be 2,121-folds.

ASSAY PROCEDURE

1. Add 50 µl of sample or standard to each test well. All standards should be tested in duplicate. Cover the plate to avoid evaporation and incubate at room temperature for 1 hour.
2. Aspirate all liquid from the test wells. Wash wells 3 times with 100 µl of wash buffer-solution-1. Remove residues by tapping the wells in an inverted position on a paper towel.
3. Add 50 µl of Enzyme-labeled Anti-apolipoprotein A1 pAb to each test well. Cover the plate to avoid evaporation and incubate at room temperature for 1 hour.
4. Aspirate all liquid from the test wells. Wash wells 4 times with 100 µl of wash buffer solution-2. Remove residues by tapping the wells in an inverted position on a paper towel.
5. Add 50 µl of substrate solution to each test well. Incubate the plate at room temperature for 10 minutes.
6. Add 50 µl of stop reagent to each test well.
7. Measure absorbance at primary wavelength of 492 nm (reference wavelength of 620–690 nm optional).

CALCULATION OF RESULTS

1. Plot the absorbance of the standards against the standard concentration on graph paper. Draw a smooth curve through these points to construct the calibration curve.
2. Read the concentrations for the diluted unknown samples from the standard curve. Calculate the concentrations for the diluted unknown samples by multiplying by the dilution factor.

PROCEDURAL NOTES

1. Standard must be reconstituted just prior to use. **The standard solution after reconstitution can not be stored.**
2. Specimens should be EDTA-plasma. **Serum can not be used.**
3. Specimens must be kept on ice immediately after the collection of blood until the plasma will be diluted with the stabilization buffer. They can not be stored at room temperature or in a refrigerator. Also, the dilution of plasma with the stabilization buffer must be performed within 6 hours.
4. The diluted sample would be stable at 2-10°C for 5 days. If the assay would not be performed within 5 days, the diluted sample should be stored at below -60°C (deep freeze).

REFERENCES

1. A new sandwich enzyme immunoassay for measurement of plasma pre- β 1-HDL levels. Miyazaki O et al. Journal of Lipid Research 2000; 41, 2083-2088.
2. Analytical performance of a sandwich enzyme immunoassay for pre- β 1-HDL in stabilized plasma. Miida T et al., J Lipid Res. 2003 Mar;44(3):645-650.