american diagnostica GmbH
SEKISUI
Kaplaneigasse 35, 64319 Pfungstadt
Tel: +49 6151 990899, Fax: +49 6151 990899

CETP ELISA
Product No. 278181

Storage: 2–10°C For Research Use Only!

INTENDED USE

The CETP ELISA kit is an *in vitro* quantitative assay for CETP (cholesteryl ester transfer protein) in human serum and plasma.

EXPLANATION OF THE TEST

Cholesteryl ester transfer protein (CETP) mediates the transfer/exchange of cholesteryl ester (CE) and triglyceride (TG) between plasma lipoproteins. Because CE is mainly generated by lecithin: cholesterol acyltransferase in HDL in plasma, the hetero-exchange of CE with TG by CETP leads to the net CE transfer from HDL to apolipoprotein B-containing lipoproteins. This reaction is believed to be one of the key steps of cholesterol transport from peripheral tissues to the liver, which is proposed to involve cellular cholesterol efflux to HDL, its esterification in HDL, CE transfer to other lipoproteins, and eventually, the uptake of the lipoproteins by the liver via receptor-mediated processes.
The pathway is of physiological importance because the cholesterol molecule is not catabolized in the peripheral tissues except for the steroidogenic cells, and thus CETP is expected to play an important role in cholesterol homeostasis.

CETP is a new therapeutic target, because the cholesteryl ester transfer process lowers HDL cholesterol and contributes to an atherogenic lipoprotein profile, particularly when plasma triglycerides are high.
Clinical evidence suggests that coronary artery calcification as well as intima media thickness is positively related to plasma cholesteryl ester transfer, and that high plasma CETP concentration is associated with increased cardiovascular risk in hypertriglyceridaemia.
However, CETP could also have anti-atherogenic potential, since it provides a potentially beneficial route for delivery of HDL-derived cholesteryl esters to the liver. In addition, CETP could also favourably stimulate peripheral cell cholesterol removal and enhance hepatic cholesterol uptake. Recent evidence suggests that a high CETP level may confer lower cardiovascular risk in the context of low triglycerides.

PRINCIPLE OF THE METHOD

Test wells are coated with anti-CETP MoAb (3-11D). CETP 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, HRP-labeled anti-CETP mAb (14-8F) 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 CETP in the sample.

REAGENTS

	Content	Component	Package
MTP	CETP-mAb coated wells	anti-CETP mAb (3-11D) coated plate	1 plate
WASH	Wash buffer concentrate (10x)	PBS (pH 7.2)	100 mL
DILB	Dilution buffer	citrate buffer (pH 5.5)	100 mL
CON	Enzyme-labeled mAb concentrate (7x)	HRP-labeled anti-CETP mAb (14-8F)	1 mL
SUB	Substrate (lyophilized)	o-phenylenediamine	2 vials
SUBB	Substrate buffer	H_2O_2 in citrate buffer (pH 5.0)	15 mL
STOP	Stop reagent	H_2SO_4 (7.7%)	10 mL
STD	Standard (lyophilized)	human plasma	1 vial

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 2-10ºC.

1. Wash buffer: Dilute the wash buffer concentrate with 900 mL of distilled water. Working wash buffer stored at 2-10ºC is stable for 1 month.
2. Enzyme-labeled antibody concentrate: Dilute Enzyme-labeled antibody concentrated with 6 mL of Dilution buffer. Working Enzyme-labeled antibody solution stored at 2-10ºC is stable for 2 weeks.
3. Substrate solution: Just prior to use, reconstitute the Substrate by adding 6 mL of Substrate buffer to the substrate vial. Since the substrate is light sensitive, it should not be exposed to excessive light. Working substrate solution should be used within 1 hour after reconstitution.
4. Standard: Reconstitute standard by adding 1.0 mL of Dilution buffer to the standard vial, which contains the stock solution of CETP. The content of CETP is indicated on the label. The stock solution of the standard is stable for 2 weeks if stored at 2-10ºC. Just prior to use, the serial dilution series should be prepared as follows to construct a standard curve.

CETP Content	a	a/2	a/4	a/8	a/16	a/32	0	µg/mL
CETP Stock sol.	150	150	150	150	150	150	0	µL
		↗	↗	↗	↗			
Dilution buffer	0	150	150	150	150	150	150	µL

5. Others: Seal extra strips with plate tape sealer and store at 2-10ºC for future use.

When stored properly at 2-10ºC, the Dilution buffer, Substrate buffer, and Stop reagent are stable until the expiration date on the label.

PREPARATION OF SAMPLES

Samples must be diluted to 1:80 with dilution buffer (Sample 10µL + Dilution buffer 800µL) before they are added to the plate. If the obtained absorbance exceeds the range of the calibration curve, dilute the sample with higher volume of Dilution buffer for another assay.

ASSAY PROCEDURE

Reagent	Vol.	Procedure
Samples or Standard	50 µl	Add sample to the center of each test well. All standards should be tested twice. Incubate the covered plate for 2 hours at room temp.
Thoroughly remove solution from wells		
Working wash buffer	350 µl	Wash wells 3 times. Thoroughly remove droplets.
Working anti-CETP MoAb HRP conjugate	50 µl	Add to each test well. Incubate the covered plate for 1 hour at room temp.
Thoroughly remove solution from wells		
Working wash buffer	350 µl	Wash well 3 times. Thoroughly remove droplets.
Working substrate solution	50 µl	Add to each test well. Incubate the covered plate for 15 minutes at room temp.
Stop reagent	50 µl	Add to each test well.
Read the absorbance of each well at 492 nm.		

CALCULATION OF RESULTS

Calculate the Δ absorbance by subtracting the absorbance of the 0 μg/mL standard from those of other standards and unknown samples. Plot the Δ absorbance of the standards against the standard concentration on log-log or semi-log graph paper. Draw a smooth curve through these points to construct the standard curve. Read the concentrations for the diluted unknown samples from the standard curve.

PROCEDURAL NOTES

1. A standard curve must be run with each assay.
2. Read absorbances just after completion of the assay.
3. The human plasma contained in the calibrator was tested and found negative for presence of Ab to HIV-1/2, the Ab to HCV, and HBs Ag.
4. Stop reagent (1.5N H_2SO_4) is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes, and clothing. If contact occurs, immediately wash the area thoroughly with water.
5. All residual wash buffer must be drained from the wells by aspiration or by decantation followed by tapping the plate forcefully on absorbent paper.

REFERENCES

1. Sasai K, Okumura-Noji K, Hibino T, Ikeuchi R, Sakuma N, Fujinami T, and Yokoyama S. Human cholesteryl ester transfer protein measured by enzyme-linked immunosorbent assay with two monoclonal antibodies against rabbit cholesteryl ester transfer protein: plasma cholesteryl ester transfer protein and lipoproteins among Japanese hypercholesterolemic patients. Clin Chem (1998) 44, 1466-1473.
2. Ko KWS, Ohnishi T, and Yokoyama S. Triglyceride transfer is required for net cholesteryl ester transfer between lipoproteins in plasma by lipid transfer protein. Evidence for a hetero-exchange transfer mechanism demonstrated by using novel monoclonal antibodies. J Biol Chem (1994) 269, 28206-28213.
3. Saito K, Kobori K, Hashimoto H, Ito S, Manabe M, and Yokoyama S. Epitope mapping for the anti-rabbit cholesteryl ester transfer protein monoclonal antibody that selectively inhibits triglyceride transfer. J Lipid Res (1999) 40, 2013-2021.

278181©ADGmbH_770118-004