APPLICATION

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

ASSAY PRINCIPLES

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 MoAb (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.

CONTENTS

Content	Component	Package
1. CETP-MoAb coated wells.	anti-CETP MoAb (3-11D) coated plate	one 96-well module plate
2. Dilution buffer	citrate buffer (pH 5.5)	1 x 100 mL
3. Enzyme-labeled MoAb concentrate (7-times)	HRP-labeled anti-CETP MoAb (14-8F)	1 mL (7-times)
4. Wash buffer concentrate (10-times)	phosphate buffer concentrate (pH 7.2)	100 mL (10-times)
5. Substrate (lyophilized)	o-phenylenediamine	2 vials
6. Substrate buffer	H_2O_2 in citrate buffer (pH 5.0)	15 mL
7. Stop reagent	H_2SO_4 (7.7%)	10 mL
8. Calibrator (lyophilized)	human plasma	1 vial

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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. Calibrator: Reconstitute Calibrator by adding 1.0 mL of Dilution buffer to the Calibrator vial, which contains the stock solution of CETP. The content of CETP is indicated on the label. The stock solution of the Calibrator 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 calibration curve.

Content of CETP	a	a/2	a/4	a/8	a/16	a/32	0	µg/mL
Stock sol. of CETP	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.

SAMPLE PREPARATION

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 exceed the range of the calibration curve, dilute the sample with higher volume of Dilution buffer for another assay.

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ADDITIONAL REQUIRED MATERIALS THAT ARE NOT PROVIEDED IN THIS KIT

1: Microplate reader capable of measurement at 492 nm.

2: 8-channel pipet covering 50-200µL.

3: 1-channel pipet covering 20-1000µL.

4: Deionized or distilled water.

5: Plastic test tube.

6: Volumetric flask of cylinder (1000 mL).

7: Absorbent paper towels.

8: Graph paper (log-log or semi-log).

9: 96-well plate dust cover or lid, if available; plastic wrap as an alternative cover.

10: Micro-plate shaker with horizontal circular movement, if available.

11: Plate washer, automated or manual, if available.

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ASSAY PROCEDURE

Reagent	Vol.	Procedure
	50µl	Add a 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	350µl	Wash wells 3 times. Thoroughly remove droplets.
Working anti-CETP MoAb HRP conjugate	50µl	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.		

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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 that when multiplied by the dilution factor gives the amount of CETP in unknown samples.

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 the 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.

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