CETP mAb (14-8F) is added. After 2
and washing to remove all of the unbound material, HRP-labeled anti-
ester transfer protein) in human serum and plasma.

is captured by the antibody in the 1
cholesterol uptake. Recent evidence suggests that a high CETP level may
enhance hepatic cholesteryl esters to the liver. In addition, CETP could also favourably
provides a potentially beneficial route for delivery of HDL-derived
cholesterol homeostasis.

However, CETP could also have anti-atherogenic potential, since it
confers lower cardiovascular risk in the context of low triglycerides. 

The pathway is of physiological importance because the cholesterol
cell cholesterol removal and transfer, and that high plasma CETP concentration is
intima media thickness is positively related to plasma cholesteryl ester
lipoproteins by the liver via receptor-mediated processes.

CETP leads to the net CE transfer from HDL to apolipoprotein B-
acyltransferase in HDL in plasma, the hetero-exchange of CE with TG by
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 cholesteryl 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.

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.

PRECAUTIONS
Source material for some of the reagents in this kit is of human origin. This
material has been tested and found to be negative for hepatitis B surface
antigen (HbsAg), antibodies to hepatitis C virus (HCV), and antibodies to
human immunodeficiency virus (HIV-1 and HIV-2). Because no known test
method can offer complete assurance that infectious agents are absent,
handle reagents and patient samples as if they are capable of transmitting
infectious disease.

WASH
STANDARD / DILUENT
Warning
H317, P280, P333+P313

SUB
Warning

STOP
Warning
H315, P280, P332 + P313, P302+P352, P362 + P364

Hazard Statements:
H303 May be harmful if swallowed.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.
H341 Suspected of causing genetic defects.
H351 Suspected of causing cancer.
H401 Toxic to aquatic life.
H411 Toxic to aquatic life with long lasting effects.

Precautionary Statements:
P202 Do not handle until all safety precautions have been read and
understood.
P261 Avoid breathing dust/fume.
P264 Wash hands thoroughly after handling.
P272 Contaminated work clothing should not be allowed out of the
workplace.
P273 Avoid release to the environment.
P280 Wear protective gloves/ eye protection/face protection.
P312 Call a POISON CENTER/doctor/physician if you feel unwell.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several
minutes. Remove contact lenses, if present and easy to do.
Continue rinsing.
P337 + P313: IF eye irritation persists: Get medical advice/attention.
P332 + P313: IF skin irritation occurs: Get medical advice/attention.
P362 + P364: Take off contaminated clothing and wash it before reuse.

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.

1. Wash buffer: Dilute the wash buffer concentrate with 900 mL of distilled
water. Working wash buffer stored at 2-10°C 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 2-10°C 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.

<table>
<thead>
<tr>
<th>CETP Content</th>
<th>a</th>
<th>a/2</th>
<th>a/4</th>
<th>a/8</th>
<th>a/16</th>
<th>a/32</th>
<th>0 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP Stock sol.</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>0 µL</td>
</tr>
<tr>
<td>Dilution buffer</td>
<td>0</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150 µL</td>
</tr>
</tbody>
</table>

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 10 µL + Dilution buffer 800 µL) 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**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Vol.</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples or Standard</td>
<td>50 µL</td>
<td>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.</td>
</tr>
<tr>
<td>Working wash buffer</td>
<td>350 µL</td>
<td>Wash wells 3 times. Thoroughly remove droplets.</td>
</tr>
<tr>
<td>Working anti-CETP MoAb HRP conjugate</td>
<td>50 µL</td>
<td>Add to each test well. Incubate the covered plate for 1 hour at room temp.</td>
</tr>
<tr>
<td>Working substrate solution</td>
<td>50 µL</td>
<td>Add to each test well. Incubate the covered plate for 15 minutes at room temp.</td>
</tr>
<tr>
<td>Stop reagent</td>
<td>50 µL</td>
<td>Add to each test well.</td>
</tr>
</tbody>
</table>

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

**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, the Ab to HCV, and HBs Ag.
4. Stop reagent (1.5N H₂SO₄) 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**


**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.