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Product Information

CETP Activity Assay Kit
Supplied by Roar Biomedical, Inc

Catalog Number **MAK106**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Cholesteryl ester transfer protein (CETP) is present in normal human plasma. The protein transfers neutral lipids from high density lipoproteins (HDL) to very low density lipoprotein (VLDL) and low density lipoprotein (LDL). CETP plays an important role in lipoprotein metabolism and influences the reverse cholesterol transport pathway. The method is useful for measuring CETP activity in plasma or serum in all species that express CETP.

The CETP Activity Assay Kit uses a proprietary substrate that enables the detection of CETP-mediated transfer of neutral lipid from the substrate to a physiological acceptor. The transfer activity results in an increase in fluorescence intensity (λ_{ex} = 465/ λ_{em} = 535 nm). In a total volume of 200 μL, the assay is linear from 0.2–0.8 μL of normal human plasma. Assay results are not affected by endogenous plasma HDL, LDL, or VLDL concentrations. Assay substrates are stable at high DMSO concentrations (up to 10% v/v); however, high DMSO concentrations affect the activity of purified CETP.

Components

The kit is sufficient for 100 assays in 200 μL total assay volume.

Component	Amount
Donor Particle (concentration on label) Catalog Number MAK106A	0.4 mL
Acceptor Particle Catalog Number MAK106B	0.4 mL
CETP Assay Buffer Catalog Number MAK106C	20 mL

Reagents and Equipment Required but Not Provided.

- 96 well U-bottom black plates for fluorescence assays.
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- 2-propanol (isopropanol, Catalog Number 34863)
- Torcetrapib (Catalog Number PZ0170), for assay validation

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening.

Storage/Stability

The kit is shipped on wet ice. Storage at 2–8 °C, protected from light, is recommended. DO NOT FREEZE. Components are stable for 1 year, if stored properly.

Assay substrates are stable at high DMSO concentration (up to10% v/v).
Note: High DMSO concentration affects the activity of purified CETP.

Procedures
All samples and standards should be run in duplicate.

Standards for Fluorometric Detection
1. Disperse 5 μL of the Donor Particle solution (Catalog Number MAK106A) into 2 mL of 2-propanol. For example, a 260 nmoles/mL Donor Particle solution prepares a 1.3 nmole/tube Stock Standard Solution. Using the Stock Standard Solution, make four serial 2-fold dilutions (1 mL of previous standard solution and 1 mL of 2-propanol), generating five tubes with decreasing amounts of fluorescent donor substrate.

2. Add 200 μL of each standard dilution to a separate well of the multiwell plate. For the example in step 1 (dilutions of a 1.3 nmole/tube Stock Standard Solution), this would generate standards of 130, 65, 32.5, 16.3, and 8.1 pmole/well. 200 μL of 2-propanol is used as the 0 (Blank) Standard. Measure the fluorescence intensity (λ_{ex} = 465/ λ_{em} = 535 nm) of each standard. The standard curve is used for calculating pmoles of substrate transferred from fluorescence intensity units.

Sample Preparation
Notes: If the CETP sample is plasma, the transfer reaction will occur without the exogenous Acceptor Particles due to endogenous acceptor plasma lipoproteins.

The CETP sample should NOT be stored at 2–8 °C. Samples should be stored at –80 °C to maintain activity. Rabbit plasma or serum has 2 to 2.5-fold the CETP activity of normal human plasma and must be kept frozen. Different plasma samples will have different CETP activity levels.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Combine 4 μL of Donor Particle solution and 4 μL of Acceptor Particle solution with the desired CETP sample (0.2–0.8 μL of undiluted plasma or serum, fresh or frozen). Bring final volume in well to 200 μL with CETP Assay Buffer. Prepare a sample blank that contains 4 μL of Donor Particle solution and 4 μL of Acceptor Particle solution and 192 μL of CETP Assay Buffer.
Notes: Donor and Acceptor Particle solutions may be mixed with buffer and pipetted as one step.

Plasma should be diluted ten-fold with buffer and then pipetted at 10× the original volume.

Assay Reaction
1. Seal plate and incubate for 3 hours at 37 °C. Linearity may be accomplished with more plasma and a shorter incubation time.
   Notes: The microplate incubator must be able to rapidly raise the assay temperature to 37 °C. Large, humidified air incubators may cause problems by slowly increasing the temperature from 25 °C to only 34 °C after three hours. Floating the plate in a water bath is recommended, rather than using an air incubator.

   Never incubate the plate in the microplate reader.

   Fluorescent assays are highly sensitive and will respond to slight changes in assay volume. Microplate incubations must be placed in a sealed container with standing water to prevent evaporation. Microplates should be sealed as tightly as possible with plate sealers.

2. Measure the increase in fluorescence of samples using a fluorometer (λ_{ex} = 465/λ_{em} = 535 nm). Determine the fluorescence intensity in the plasma or serum samples by subtracting the fluorescence intensity of the sample blank from each sample.
   Note: The fluorescence of the sample blank should **NOT** increase over time. It is normal for the sample blank to become slightly lower in intensity in the first 15 minutes, but never higher.

Validation Assay Reaction

Torcetrapib is a cholesteryl ester transfer protein (CETP) inhibitor.

1. Dissolve torcetrapib, 6.00 mg, in 1 mL of freshly opened DMSO to generate a 10.00 mM Torcetrapib Stock Solution.
   Note: Give extra consideration to the compound's hydrophobic properties and the tendency of DMSO to absorb water from the atmosphere.

2. Dilute the 10.00 mM Torcetrapib Stock Solution 100-fold by adding 5 μL of the Torcetrapib Stock Solution to 495 μL of DMSO generating a 100.0 μM solution.

3. Add 50 μL of the 100.0 μM solution to 750 μL of DMSO to prepare a Torcetrapib Working Solution of 6.25 μM. Serial dilutions are made to give the following concentrations: 6.25, 3.12, 1.56, 0.78, and 0.078 μM. Use DMSO as 0 μM (inhibitor blank).

4. Dilute plasma sample 10-fold with buffer and store on ice.

5. Prepare Master Reaction Mix according to Table 1 (188 μL of Master Reaction Mix is required for each reaction well). Pipette 188 μL of the Master Reaction Mix into each reaction well of a black plate.

Table 1.
Master Reaction Mix

Reagent	Volume
CETP Assay Buffer	180 μL
Donor Particle	4 μL
Acceptor Particle	4 μL

6. Add 2 μL of each serial dilution of the Torcetrapib Working Solution and the inhibitor blank to separate wells, mixing well by pipetting. Add 10 μL of the diluted plasma, again mixing well by pipetting. This will give final torcetrapib concentrations of 62.5, 31.2, 15.6, 7.8, 0.78, and 0 nM per well.

7. For a sample blank, add 10 μL of buffer and 2 μL of DMSO, in place of torcetrapib and sample solutions.
   Note: It is suggested to run negative control with torcetrapib solutions and buffer (in place of plasma sample) to make certain results are not an artifact from destruction of the donor/acceptor particles or a DMSO effect. The assay tolerates up to 10% DMSO.

8. Seal plate and incubate for 3 hours at 37 °C.

9. Measure the increase in fluorescence of samples using a fluorimeter (λ_{ex} = 465/λ_{em} = 535 nm). Determine the fluorescence intensity in the plasma or serum samples by subtracting the sample blank fluorescence intensity from each sample.

Results

Temperature is a possible cause of difficulty in obtaining reproducible results. CETP activity will be reduced at temperatures below 37 °C. IC_{50} results will be impacted by temperature.

Multiple assay results from the same sample should be tight. Variability indicates evaporation, inaccurate pipetting, or incomplete mixing of assay components.

Assay results may be expressed in terms of pmoles of fluorescent substrate transferred.

Note: The substrate concentration of the Donor Particle solution (nmoles/mL) is printed on the label of the donor particle vial.

Calculate the pmoles of fluorescent substrate transferred from the standard curve using the fluorescence intensity values in the assay. Be sure to subtract the sample blank fluorescence intensity from the sample fluorescence intensity before attempting to enter the values into the regression or the values from the assay will be higher than the standard.

Human Plasma Assay

Table 2.
Typical Standard Curve

pmoles	Fluorescence Intensity duplicates		Average of duplicates minus 0 (Blank)	Regression Statistics	
130	1873	1987	1619	Multiple R	0.999677
65	1145	1162	842	R^2	0.999355
32.5	730	758	433	Adjusted R^2	0.999193
16.25	489	557	212	Intercept	20
8.1	467	423	134	X Variable 1	12.4
0 (Blank)	313	310	0		

5 μL of Donor Particle solution dispersed in 2 mL of 2-propanol. Mixture is serially diluted 2-fold and 200 μL of each dilution is read. Concentration of Donor Particle solution = 260 nmoles/mL

Table 3.
Typical Sample Values

Plasma Samples	Fluorescence Intensity, triplicates			Average	Average of Fluorescence Intensity minus Sample Blank	pmole transferred
Sample Blank	1005	1035	1026	1022		
Sample 1	2094	2113	2065	2091	1069	84.6
Sample 2	1849	1730	1738	1772	750	58.9
Sample 3	1611	1652	1652	1638	616	48

Human plasma samples assayed with CETP activity kit. The plasma samples were incubated for 3 hours at 37 °C in a total assay volume of 200 μL (mixture of 4 μL each of Donor and Acceptor Particle solutions and 187 μL of buffer with 5 μL of 10-fold diluted plasma).

Figure 1.
CETP Activity in human plasma

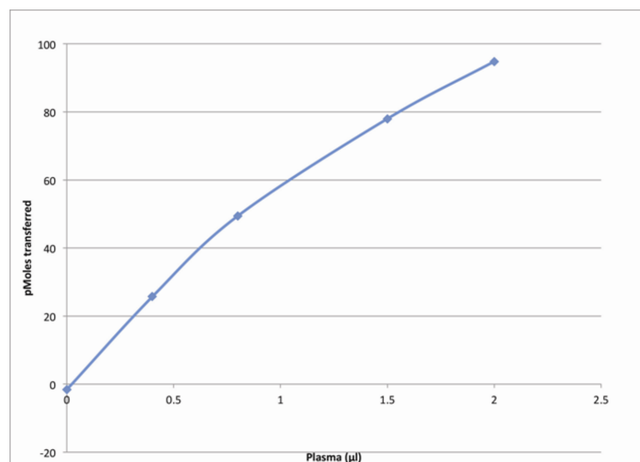
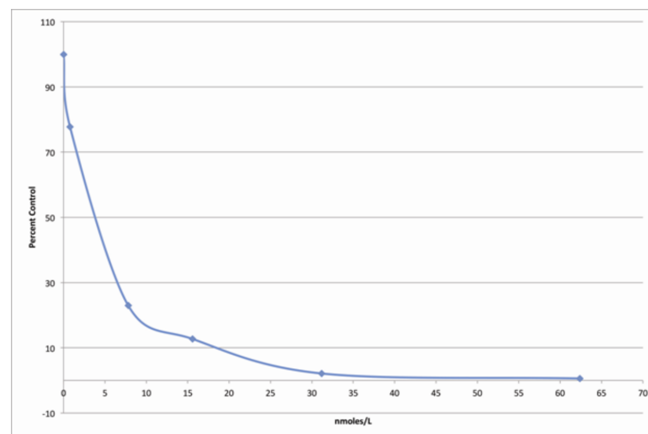


Figure 2.
Torcetrapib Inhibition of CETP Activity



Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

This product is supplied by Roar Biomedical, Inc. and covered by several patents including U.S. Pat. Nos. 5,585,235; 5,618,683; 5,770,355, and related US and foreign patents.

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