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Product Information

CETP Activity Assay Kit

Catalog Number **MAK042**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Cholesteryl ester transfer protein (CETP) also known as plasma lipid transfer protein, is a liver protein that facilitates the transfer of cholesteryl esters and triglycerides from high density lipoprotein (HDL) to low density lipoprotein (LDL).

The CETP Activity Assay kit provides a simple and direct procedure for measuring CETP activity in serum or plasma samples. CETP activity is determined using a donor molecule containing a self-quenched fluorescent neutral lipid, which is transferred to an acceptor molecule in the presence of CETP. CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence (λ_{ex} = 465/λ_{em} = 535 nm), proportional to the CETP activity present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Donor Molecule (0.1 mM labeled lipids) Catalog Number MAK042A	1 mL
Acceptor Molecule Catalog Number MAK042B	1 mL
CETP Assay Buffer, 10× Catalog Number MAK042C	5 mL
Positive Control (Rabbit Serum) Catalog Number MAK042D	30 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader.
- Isopropanol (Catalog Number I9516 or equivalent).

Precautions and Disclaimer

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

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

CETP Assay Buffer – Allow buffer to come to room temperature before use.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>Standard Curve</u>

Prepare 6 tubes labeled T0 to T5. Add 0.2 mL of isopropanol to tubes T0 to T4. Add 0.4 mL of isopropanol to T5. Add 2 µL of the Donor Molecule solution, containing 0.1 mM labeled lipids, into T5 and vortex to mix. Transfer 0.2 mL from T5 to T4. Mix and then transfer 0.2 mL from T4 to T3. Mix and then transfer 0.2 mL from T3 to T2. Mix and then transfer 0.2 mL from T2 to T1. Transfer 0.2 mL of each standard to a 96 well plate, generating 6.25, 12.5, 25, 50, and 100 pmole/well standards. Use isoproanol as the 0 (blank). In isopropanol the fluorescence of the lipid is no longer quenched and the measured fluorescence is proportional to the lipid present. Measure fluorescence intensity (λ_{ex} = 465/λ_{em} = 535 nm).

Assay Reaction

1. Bring serum or plasma samples (1–5 µL) up to a final volume of 100 µL with water. Mix well and transfer samples to the wells. As a background control (water control), transfer 100 µL of water to a well.
   Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

   For the positive control (optional), bring 1–3 µL of the rabbit serum positive control up to a final volume of 100 µL with water. Mix well and transfer to a well.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Donor Molecule	10 µL
Acceptor Molecule	10 µL
CETP Assay Buffer, 10×	20 µL
Water	60 µL

2. Add 100 µL of the Master Reaction Mix to each of the control and sample wells. Mix well using a horizontal shaker or by pipetting.

3. Seal the plate tightly and incubate the plate at 37 °C for 30–60 minutes. Protect the plate from light during the incubation.

4. Measure fluorescence intensity (λ_{ex} = 465/ λ_{em} = 535 nm).

Results

Calculations

Correct for the background by subtracting the 0 (isopropanol blank) value from values obtained for the standards. Use the corrected values of the standards to plot the standard curve.
Note: A new standard curve must be set up each time the assay is run.

Correct for the assay background by subtracting the background control (water control) value from the positive control and sample readings. This background value can be significant and must be subtracted from the positive control and sample readings. Determine the amount of lipid transferred during the CETP reaction by comparison to the standard curve.

The CETP activity of a sample may be determined by the following equation:

$$\text{CETP Activity} = \frac{\text{Amount of Lipids Transferred}}{(\text{Reaction Time}) \times V}$$

Amount of Lipids Transferred = determined from standard curve
Reaction Time = in hours
V = sample volume (µL) added to well

Example:
Amount of Lipids Transferred = 95.95 pmole
Reaction Time = 1 hour
V (sample volume) = 3 µL

$$\text{CETP Activity} = \frac{95.95 \text{ pmole}}{(1 \text{ hr}) \times 3 \text{ µL}}$$

CETP Activity = 31.98 pmole/µL/hr

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates with clear bottoms
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 needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	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
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to 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

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