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Product Information

CETP RP Activity Assay
Supplied by Roar Biomedical, Inc.

Catalog Number **MAK305**
Storage Temperature 2–8 °C; DO NOT FREEZE

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 lipoproteins (VLDL) and low density lipoproteins (LDL). CETP plays an important role in lipoprotein metabolism and influences the reverse cholesterol transport pathway.

The CETP RP Activity Assay uses a proprietary substrate that enables the detection of CETP-mediated neutral lipid mass transfer. The method is useful for measuring CETP activity in recombinant protein (RP) or purified CETP samples and has a high DMSO tolerance. The transfer activity results in an increase in fluorescence intensity (λ_{ex} = 465/λ_{em} = 535 nm). With recombinant CETP as the CETP source, the assay is linear over a wide range of CETP concentrations and provides very high signal-to-background values. Typical Z factor: ~0.9.

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

CETP RP Donor Particle Catalog Number MAK305A	400 µL
CETP RP Acceptor Particle Catalog Number MAK305B	400 µL
CETP RP Assay Buffer Catalog Number MAK305C	20 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plates. It is recommended to use black U-bottom plates.
- Adhesive plate seals
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- 2-propanol (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 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 2 years if stored properly.

Procedure
All samples and standards should be run in duplicate.

Standard Curve for Fluorometric Detection
Notes: A new standard curve must be set up each time the assay is run.

Do not incubate standards before reading.

The fluorescent substrate concentration (nmoles/mL) of the CETP RP Donor Particle is printed on the label of the vial. Based on the CETP RP Donor Particle concentration on the label, accurately calculate the dilution needed to prepare the 0.65 nmole/mL (650 pmoles/mL) Donor Particle Stock Solution.

1. Add 5 µL of the CETP RP Donor Particle to sufficient 2-propanol to prepare a 0.65 nmole/mL (650 pmoles/mL) Donor Particle Stock Solution based on the concentration listed on the label.

2. Perform 5 serial dilutions (1:1 of the Donor Particle Stock Solution to 2-propanol) starting with 1 mL of the 650 pmoles/mL Donor Particle Stock Solution. This results in prepared concentrations of 650, 325, 162.5, 81.3, 40.6, and 20.3 pmoles/mL.

3. Add 200 µL of each prepared concentration to separate wells of the multiwell plate, generating standards of 130, 65, 32.5, 16.3, 8.1, and 4.1 pmoles/well. For the 0 pmole (blank) standard, use 200 µL of 2-propanol.

4. Measure the fluorescence intensity of the standards and blank using a fluorometer (λ_{ex} = 465/ λ_{em} = 535 nm). Do not incubate standards before reading.

Sample Preparation
The CETP sample should not be stored at 2–8 °C. Samples should be stored at –80 °C to maintain activity.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. Dilute samples in CETP RP Assay Buffer if necessary.

1. Add 1–100 µL of CETP sample per well. Bring samples to a final volume of 100 µL with CETP RP Assay Buffer.

2. Prepare a Master Mix containing 92 µL of CETP RP Assay Buffer, 4 µL of CETP RP Donor Particle solution, and 4 µL of CETP RP Acceptor Particle solution per sample well. 100 µL of Master Mix is required for each reaction (well).

3. Add 100 µL of the Master Mix to each well. Mix well using a horizontal shaker or by pipetting.

4. Prepare duplicate wells for the sample blank, each with 100 µL of Master Mix and 100 µL of CETP RP Assay Buffer.

Assay Reaction
Notes: If desired, the assay can be read at multiple time points.

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.

1. Seal plate with an adhesive plate seal and incubate for 1–3 hours at 37 °C.

   Notes: Use of a water bath is recommended, as the incubator must be able to rapidly raise the assay temperature to 37 °C. Large humidified air incubators may not increase the temperature quickly enough. Do not incubate the plate in the microplate reader.

   Incubations must be sealed to prevent evaporation, as fluorescent assays are highly sensitive and will respond to slight changes in assay volume.

2. Measure the fluorescence intensity of the samples and the blank using a fluorometer (λ_{ex} = 465/ λ_{em} = 535 nm).

Results
Notes: 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.

Variability indicates evaporation, inaccurate pipetting, or incomplete mixing of assay components.

Calculations
All measurements refer to the average values calculated for sample and standard duplicates.

1. To construct a standard curve, obtain the corrected fluorescence measurements by subtracting the background fluorescence intensity of the 0 (blank) standard from the readings taken for the standards. Use the corrected values to plot a standard curve.

2. Subtract the fluorescence intensity of the sample blank from the sample measurements to obtain corrected fluorescence measurements for the samples. Using the corrected fluorescence sample measurements and the standard curve, determine the pmoles of substrate transferred for the samples.

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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