



## Product Information

*Revised: August 2006*

### Roar CETP RP Activity Assay Kit, 250 assays

Catalog No. RB-RPAK [U.S. Pat. Nos. 5,585,235; 5,618,683; 5,770,355; and pats. pend.]

<b>Assay Method:</b>	Fluorometric
<b>Number of Assays:</b>	250 assays in 200 µl total assay volume
<b>Kit Contents:</b>	Donor particle: 1 ml Acceptor particle: 1 ml Assay buffer: 5 ml 10X
<b>Storage and Handling:</b>	Store kit components at 4°C. If stored properly, components are stable for up to 1 year. DO NOT FREEZE.
<b>Instrumentation:</b>	Fluorescence spectrophotometer: cuvette or microplate reading formats Excitation: 465 nm / Emission: 535 nm

## Introduction

Cholesteryl ester transfer protein (CETP) is present in normal human plasma and 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 **Roar CETP RP Activity Assay** is useful for measuring changes in CETP activity for inhibitor discovery and structure activity studies.

The **Roar CETP RP Activity Assay Kit** uses proprietary substrates that enable the detection of specific CETP-mediated transfer of neutral lipid from a donor substrate to a synthetic acceptor. Transfer activity results in an increase in fluorescence intensity. The CETP RP assay is linear over a wide range of CETP concentrations and provides very high signal-to-background values. The assay z-factor is 0.9 and the pre-mixed reagents are stable for longer than 48 hours when stored at 4°C.

## Advantages

- Synthetic assay components support manipulation for mechanism of action studies.
- Unusually high signal-to-background with linearity.
- Assay components are stable for up to 1 year.
- DMSO tolerance > 10% (v/v).

## Materials Required, But Not Supplied

- Fluorimeter with appropriate wavelength capabilities (Ex: 465 nm; Em: 535 nm)
- 37°C water bath / incubator
- Recombinant or purified CETP source

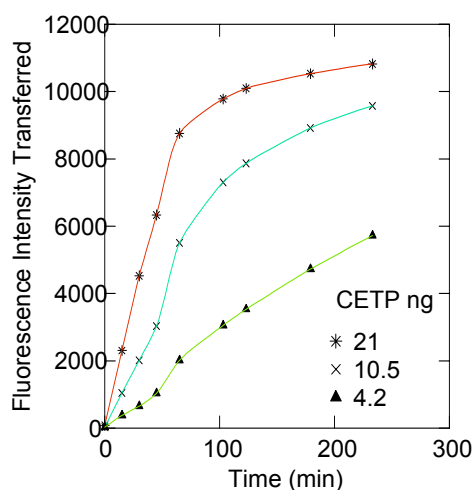
Roar RB-RPAK Activity Assay

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS OR CLINICAL DIAGNOSIS.

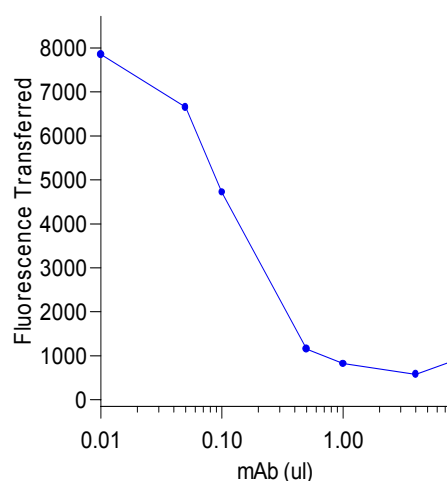
## Assay Method

1. Using the assay buffer provided with the kit, reconstitute the buffer with distilled water (5 ml 10X assay buffer plus 45 ml dH<sub>2</sub>O) to yield 50 ml of 1X assay buffer (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.4).
2. Combine 4 µl of donor particle and 4 µl of acceptor particle with CETP source in 200 µl total volume with reconstituted assay buffer. Prepare a blank that contains 4 µl of donor particle and 4 µl of acceptor particle in 200 µl total volume of assay buffer.
3. Incubate for 1 to 3 hours at 37°C.
4. Measure the increase in fluorescence of samples using a fluorimeter (excitation: 465 nm; emission: 535 nm). Determine the fluorescence intensity transferred in the assays by subtracting the blank fluorescence intensity from each sample.
5. Assay results may be expressed in terms of pmoles of fluorescent substrate transferred. The substrate concentration of the donor is provided in nmoles/ml and printed on the kit insert. For example, **Donor fluorescent substrate concentration: 260 nmoles/ml**.
6. Take 5 µl of donor (0.005 ml x 260 nmoles/ml = 1.3 nmoles) and add it to 2 ml isopropanol. Then make four 1:2 serial dilutions of the donor / isopropanol mix. There should now be five tubes with decreasing concentrations of fluorescent donor substrate. This is the standard curve for pmoles of substrate per fluorescence intensity units.
7. Measure the fluorescence in 200 µl of each dilution to develop the standard curve. The pmole amounts are 130, 65, 32.5, 16.3, 8.1 and 4.1, in this example. Calculate the pmoles transferred from this standard using the values of fluorescence transferred from your samples in the assay.

## Performance Characteristics of the Roar CETP RP Activity Kit



**Figure 1.** Recombinant CETP activity



**Figure 2.** Inhibition with CETP neutralizing monoclonal antibody

## Technical Tips

- Donor and acceptor may be mixed with buffer and pipetted as one step.
- The assay blank should **NOT** increase in fluorescence over time. It is normal for the blank to become slightly lower in intensity in the first 15 minutes, but never higher.
- Results from duplicate samples should be tight. Variability indicates evaporation, inaccurate pipetting or incomplete mixing of assay components.
- Fluorescent assays are highly sensitive and will respond to slight changes in assay volume. Microplate incubations must be sealed to prevent evaporation.
- The incubator must be able to rapidly raise the assay temperature to 37°C. Large, humidified air incubators cause problems by slowly increasing the assay temperature. **WE RECOMMEND FLOATING PLATES IN A WATER BATH RATHER THAN USING AN AIR INCUBATOR.** If you are having difficulty reproducing results, temperature is likely the cause. IC<sub>50</sub> results will be affected by temperature - CETP activity will be reduced at temperatures below 37°C.
- Microplates must be compatible with fluorescent assays. Some clear plates contain fluorescent plastic. We recommend black microplates (top-reading plate readers only).
- Filters must be within specifications. An excitation filter of 485 nm with a 20 nm bandwidth may **NOT** be used.

## Contact Information

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