Roar

Product Information

Roar CETP RP Activity Assay Kit, 100 assays

Catalog No. RB-RPAK

U.S. Pat. Nos. 5,585,235; 5,618,683; 5,770,355

Assay Method:	Fluorometric
Number of Assays:	100 assays in 200 µl total assay volume
Kit Contents:	Donor particle: 400 µl Acceptor particle: 400 µl Assay buffer: 20 ml
Storage and Handling:	Store kit components at 4°C. If stored properly, components are stable for up to 1 year. DO NOT FREEZE.
Instrumentation:	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** uses synthetic particle substrates designed for detecting changes in specific neutral-lipid-mass transfer. 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.

Applications for this method include high-throughput screening, mechanism of action studies and structure-activity relationship (SAR) work.

Advantages

- Synthetic assay components support manipulation for mechanism of action studies
- Unusually high signal-to-background with linearity
- Assay components stable for up to 1 year
- High DMSO tolerance

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

Assay Method

1. 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.

2. Incubate for 1 to 3 hours at 37°C.

3. 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.

4. 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 label of the donor particle vial. For example, **Donor fluorescent substrate concentration: 260 nmoles/ml.**

5. 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.

6. 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.

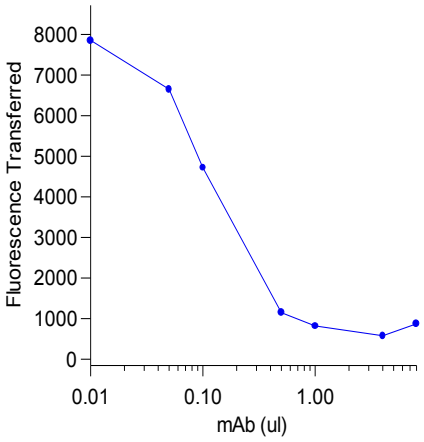

Figure 2. Inhibition with CETP neutralizing monoclonal antibody

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_{50} 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.

Related Products

R8899	Cholesteryl Ester Transfer Protein (CETP), recombinant protein
RB-CETP	Roar CETP Activity Assay Kit, 250 assays
RB-EVAK	Roar Ex Vivo CETP Activity Assay Kit, 92 assays

RB-CETP Cited Reference

Roar CETP Activity Assay Kit

Wade L, Nadeem N, Young IS, et al. Alpha-tocopherol induces proatherogenic changes to HDL2 & HDL3: An in vitro and ex vivo investigation. Atherosclerosis. **2012** [Article in Press].

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