



## Product Information

### Roar CETP Activity Assay Kit, 100 assays

Catalog No. RB-CETP

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

<b>Assay Method:</b>	Fluorometric
<b>Number of Assays:</b>	100 assays in 200 µl total assay volume
<b>Kit Contents:</b>	Donor particle: 400 µl Acceptor particle: 400 µl Assay buffer: 20 ml
<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. 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 **Roar 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. In a total volume of 200 µl, the assay is linear from 0.2 to 0.8 µl of normal human plasma.

### Advantages

- Assay results not affected by endogenous plasma HDL, LDL or VLDL concentrations:
  - The Roar donor particle is the preferred substrate by CETP over HDL, thus eliminating competition from endogenous HDL present in the plasma sample.
  - The addition of excess exogenous acceptor normalizes endogenous acceptor lipoprotein concentration present in the sample.
  - Other methods, including radioisotopic methods, are affected by endogenous HDL concentration. An increasing plasma HDL concentration in the sample decreases the specific activity of the labeled HDL substrate due to the equal preference by CETP for either labeled or unlabeled HDL.
- Intra- and interassay coefficients of variation: < 3% (Schierer, 2012; Harangi, 2009; Seiler, 2008; Kassai, 2007; Hudgins, 2003)
- Assay components stable for up to 1 year.
- Assay substrates stable at high DMSO concentration (>10% v/v). **Note:** High DMSO concentration affects the activity of purified CETP.

## Materials Required, But Not Supplied

- Fluorimeter with appropriate wavelength capabilities (Ex: 465 nm; Em: 535 nm)
- 37°C water bath / incubator
- CETP source: plasma / serum (fresh or frozen), recombinant or purified CETP

## Assay Method

1. Combine 4  $\mu$ l of donor particle and 4  $\mu$ l of acceptor particle with the desired CETP source (0.2 – 0.8  $\mu$ l of undiluted plasma or serum, fresh or frozen) in 200  $\mu$ l total volume with assay buffer. Prepare a blank that contains 4  $\mu$ l of donor particle and 4  $\mu$ l of acceptor particle in 200  $\mu$ l total volume of assay buffer.
2. Incubate for 3 hours at 37°C. Linearity may be accomplished with more plasma and a shorter incubation time.
3. Measure the increase in fluorescence of samples using a fluorimeter (excitation: 465 nm; emission: 535 nm). Determine the fluorescence intensity transferred in the plasma or serum samples 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. Disperse 5  $\mu$ l of donor (0.005 ml x 260 nmoles/ml = 1.3 nmoles) in 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 calculating pmoles of substrate transferred from fluorescence intensity units transferred.
6. Measure the fluorescence in 200  $\mu$ l 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. Please be sure to subtract the buffer blank fluorescence intensity from your sample fluorescence intensity before attempting to enter the values into your regression or the values from the assay will be higher than the standard.

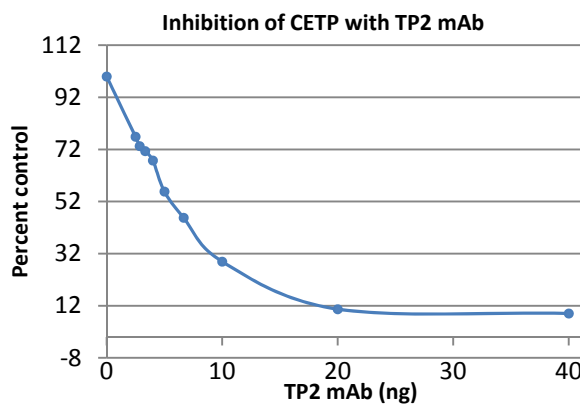
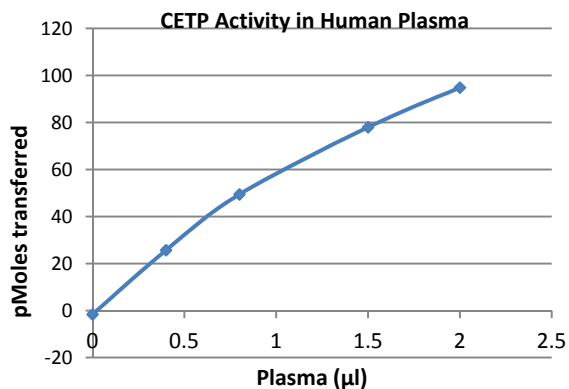
## Notes

1. The CETP source should **NOT** be stored at 4°C. Samples should be stored at -80°C to maintain activity. Rabbit plasma or serum has 2 to 2.5 times the CETP activity of normal human plasma and must be kept frozen.
2. 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.
3. Be sure to use a water bath incubator. The microplate should be allowed to float in the water bath. We recommend U-bottom, black microplates (Thermo Scientific #7205 or #7005). These plates have round, not flat, bottom wells and water will surround the wells during incubation. See also the **Technical Tips** section below for more on this topic.

### Things to remember:

- If you are having difficulty reproducing results, temperature is likely the cause.
- IC<sub>50</sub> results will be impacted by temperature.
- CETP activity will be reduced at temperatures below 37°C.
- Different patient plasma samples will have different CETP activity levels.

## Plasma CETP Activity Titration and Antibody Inhibition



### Human Plasma Assay Results

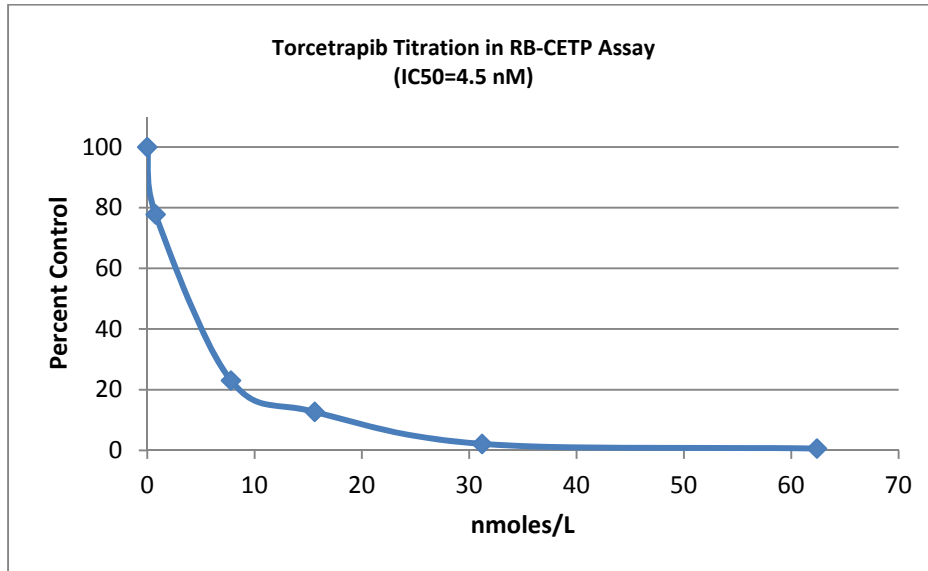
**Example:** Human plasma samples in the Roar CETP activity kit. The plasma samples were incubated for 3 hours at 37° C in a total assay volume of 200 µl (4 µl donor + 4 µl acceptor + 187 µl buffer + 5 µl 1:10 plasma dilution.)

Plasma samples	Raw fluorescence intensity x 3			Average	Fluorescence transferred	pmoles transferred
BLANK	1005	1035	1026	1022		
Human 1	2094	2113	2065	2091	1069	84
Human 2	1849	1730	1738	1772	750	58
Human 3	1611	1652	1652	1638	616	47
Human 4	1875	1879	1816	1857	835	65
Human 5	2000	2032	1991	2008	986	77
Human 6	2009	1919	2051	1993	971	76

**Standard Curve:** 5 µl donor particle dispersed in 2 ml isopropanol is serially diluted 4X and 200 µl of each dilution is read. Donor concentration = 249 nmoles/ml

pmoles / 200 µl	Fluorescence intensity units			Average FIU	Regression Statistics	
124.5	1578	1552	1565		Multiple R	0.9997933
62.3	809	811	810		R Square	0.9995867
31.1	414	432	423		Adjusted R Square	0.9994833
15.6	229	276	252.5		Intercept	41.0
7.8	124	133	128.5		X Variable 1	12.3
0	22	35	28.5			

## Assay Validation with the CETP Inhibitor Torcetrapib (Sigma #PZ0170)



### Preparation of Torcetrapib in DMSO

Torcetrapib, 5.99 mg /Sigma #PZ0170, was dissolved in 1 ml of freshly opened DMSO providing a 9.99 mM Stock Solution (extra consideration was given because of the compound's hydrophobic properties and the tendency of DMSO to absorb water from the atmosphere.) The Stock Solution was further diluted 100-fold to 99.9  $\mu$ M. Next, 50  $\mu$ l of the 99.9  $\mu$ M solution was added to 750  $\mu$ l of DMSO to give a Working Solution of torcetrapib at 6.24  $\mu$ M. Dilutions were made to give the following concentrations for the titration:

6.24 3.12 1.56 0.78 0.078 0  $\mu$ M

### Overview of the Validation Assay

Make a 1:10 dilution of plasma with buffer and store on ice. Mix the assay reagents (180  $\mu$ l buffer/ 4  $\mu$ l donor/ 4  $\mu$ l acceptor per assay) and pipet 188  $\mu$ l of the mix into the wells of a black plate. Next, add 2  $\mu$ l of each torcetrapib dilution to the wells (mix by aspiration/dispense with pipet) and then add 10  $\mu$ l of the diluted plasma and mix by aspiration/dispense with pipet. Don't forget to add a blank with just 10  $\mu$ l of buffer and 2  $\mu$ l DMSO.

The assays may also be run with buffer and torcetrapib to make sure that the result is not an artifact from destruction of the donor/acceptor particles or a DMSO effect. The assay tolerates up to 10% DMSO.

Adding 2  $\mu$ l of each torcetrapib serial dilution will give a final concentration of:

62.4 31.2 15.6 7.8 0.78 0  $\mu$ M

Seal the plate and incubate at 37°C for 3 hours. Read the plate at 465 nm excitation / 535 nm emission wavelengths.

## Technical Tips

- Donor and acceptor may be mixed with buffer and pipetted as one step. Plasma should be diluted ten-fold and then pipetted at 10x the volume.
- If plasma is used as the CETP source, transfer will occur without exogenous acceptor due to endogenous plasma lipoproteins.
- 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 - **BE SURE TO CAP TUBES**. 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.
- The microplate incubator must be able to rapidly raise the assay temperature to 37°C. Test the incubator with a small container of water and a thermometer. Large, humidified air incubators may cause problems by slowly increasing the temperature from 25° to only 34° after three hours. **WE RECOMMEND FLOATING THE PLATE IN A WATER BATH RATHER THAN USING AN AIR INCUBATOR.** See also: **Notes**.
- Never incubate the plate in the microplate reader.
- Microplates must be compatible with fluorescent assays. Some clear plates contain fluorescent plastic. We recommend black microplates (top-reading plate readers only) such as these: Thermo Scientific, black, U-bottom (Thermo #7205 or #7005).
- The filter must be within specifications. An excitation filter of 485 nm with a 20 nm bandwidth may **NOT** be used. This filter will incompletely excite the label and the standard curve will appear to work, but protein activity results will be low.

## Related Products

Cat. #R8899	CETP, partially purified, active human recombinant (rCETP), 1 ml
Cat. #RB-RPAK	Roar CETP/RP Activity Assay Kit, 250 assays
Cat. #RB-EVAK	Roar Ex Vivo CETP Activity Assay Kit, 92 assays

**For Research Use Only. Not for Diagnostic or Therapeutic Purposes.**

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