

3. **Measurement:** Preincubate the plate for 10 min at 37°C **protected from light** to allow the reaction temperature to equilibrate. Measure fluorescence (Ex/Em = 488/523 nm) in kinetic mode for 1-3 hr at 37°C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample.

Note: Samples with very high activity (such as rabbit serum), may show a decrease in rate after 1 hr.

4. **Calculation:** Subtract the zero Standard (0 pmole/well) reading from all of the other solubilized Donor Molecule Standard readings. Plot the Background-subtracted values and calculate the slope of the Standard Curve. For the Test Sample reaction wells, choose two time points (T_1 and T_2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points and subtract the Reagent Background Control reading from Sample reading:

$$RFU_1 = RFU_{1S} - RFU_{BC}$$

$$RFU_2 = RFU_{2S} - RFU_{BC}$$

Where: RFU_{1S} and RFU_{2S} are the Sample readings at time T_1 and T_2 , respectively
 RFU_{BC} and RFU_{2BC} are the Reagent Background Control readings at time T_1 and T_2 , respectively

Calculate the CETP activity of the Samples $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the Standard Curve to get B pmole of cholesteryl ester transferred by CETP during the reaction time ($\Delta T = T_2 - T_1$). Calculate Sample's CETP activity by using the following equation:

$$\text{Sample CETP Activity} = \frac{B}{\Delta T \times V} \times D = \text{pmole/hr/}\mu\text{l}$$

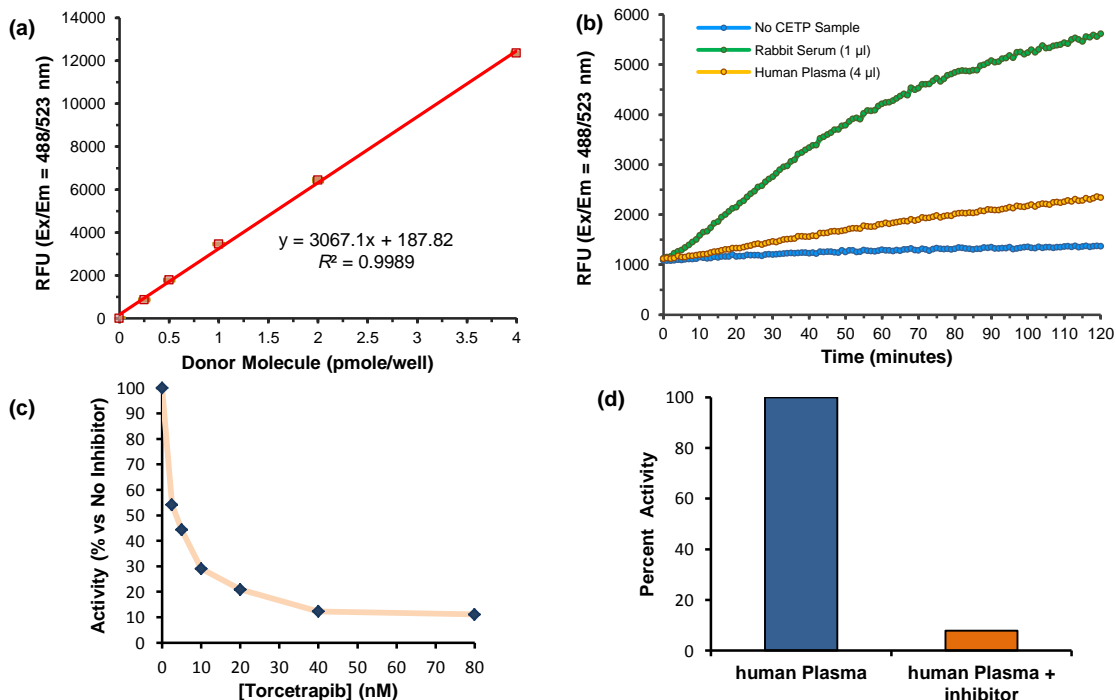
Where: B is the amount of solubilized Donor Molecule from the standard curve (in pmole)

V is the Sample volume added into the reaction well (in μl)

ΔT is the reaction time (in hours)

D is the Sample dilution factor (if applicable)

Unit Definition: One unit of CETP is the amount of protein that facilitates the transfer of 1 μmole of cholesteryl ester from the donor molecule per hour at 37°C.



Figures: (a) Solubilized Donor Molecule Standard Curve. One mole of Donor Molecule Standard corresponds to the unquenched fluorescence of cholesteryl ester analogue molecules released upon solubilization of one mole of Donor Molecule in 100% isopropanol. (b) Reaction kinetics of CETP activity in rabbit serum (1 μl) and human plasma (4 μl). (c) Inhibition of CETP activity from rabbit serum (1 μl /well) by Torcetrapib. The assay was run for 1 hour and the IC_{50} was determined to be 3.56 nM. (d) Inhibition of CETP activity from human plasma using 80 nM Torcetrapib (assay was run for 2 hours).

VIII. Related Products:

CETP Inhibitor Screening Kit II (Fluorometric) (K594)
CETP Antibody (3413)
CETP Polyclonal Antibody (6606)
EZSolution™ Dalcetrapib (2532)
Active Recombinant Human CETP (7606)
Lipoproteins, Human Plasma, High Density (4934)

HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613)
CETP Blocking Peptide (3413BP)
Dalcetrapib (2419)
Anacetrapib (2418)
Torcetrapib (2420)
Rabbit Serum (1267)

FOR RESEARCH USE ONLY! Not to be used on humans.