# ab196995
# CETP Activity Assay Kit II (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of CETP activity in animal plasma and serum.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5a, Last Updated 21 August 2023

# Table of Contents

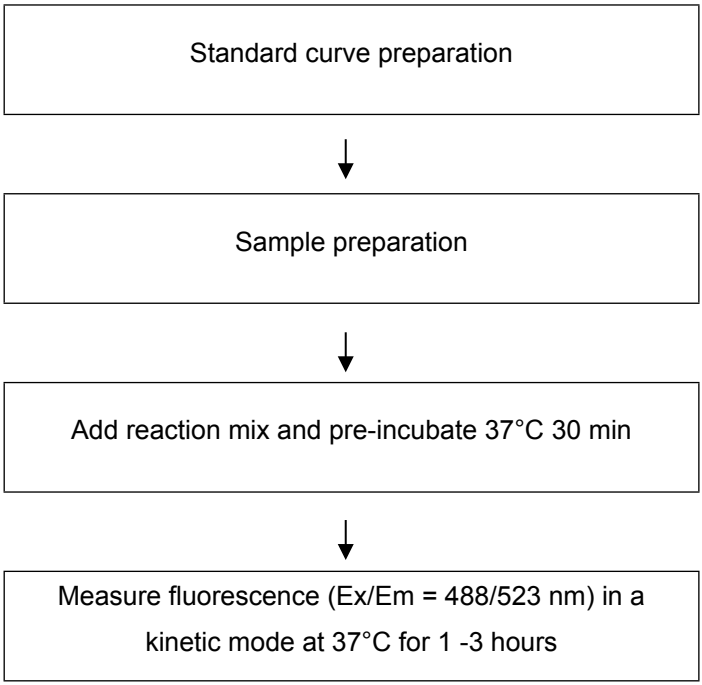
## 1. BACKGROUND

CETP Activity Assay Kit II (Fluorometric) (ab196995) uses a donor molecule containing a fluorescent self-quenched phospholipid that is transferred to an acceptor molecule in the presence of CETP. CETP-mediated transfer of the fluorescent phospholipid to the acceptor molecule results in an increase in fluorescence (Ex/Em = 488/523 nm). The fluorometric intensity is directly proportional to the amount of phospholipid transferred. The kit contains rabbit serum and torcetrapib (CETP inhibitor) as positive and negative control, respectively, for assay validation.

This assay can measure PLTP activity in plasma and serum but can also be used for testing activity of recombinant CETP protein.

Cholesteryl ester transfer protein (CETP) is a member of the lipid transfer/lipopolysaccharide binding protein gene family. CETP is plasma protein that transfers a cholesteryl ester from HDL to LDL or VLDL in exchange for a triglyceride. HDL plays an important role in lipid metabolism and cardiovascular health. HDL transports cholesterol to the liver for excretion or to steroidogenic tissues for steroid synthesis. HDL also plays an important role in the reverse cholesterol transport pathway, removing cholesterol from lipid-filled macrophages, protecting against atherosclerosis. Because of this function, CETP is viewed as a target to increase HDL, with CETP inhibition an active area of research and several CETP inhibitors at various stages of drug development.

## 2. ASSAY SUMMARY




**For kinetic mode detection, incubation time given in this summary is for guidance only.*

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 4ºC in the dark immediately upon receipt, except from the CETP Positive Control which should be stored at -80°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

## 5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) | Storage Condition (After Preparation) |
|---|---|---|---|
| Assay Buffer LIII/CETP Assay Buffer | 20 mL | 4°C | 4°C |
| CETP Donor Molecule/CETP Donor Molecule (8 nmol/mL) | 500 µL | 4°C | 4°C |
| Acceptor Molecule (CETP)/CETP Acceptor Molecule | 500 µL | 4°C | 4°C |
| CETP Positive Control/CETP Positive Control (rabbit serum) | 100 µL | -80°C | -80°C |
| Torcetrapib Solution/CETP Inhibitor (1 mM) (Torcetrapib) | 10 µL | 4°C | 4°C |

## 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- 100% isopropanol
- DMSO
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 488/523 nm
- 96 well plate: white or black plates for fluorometric assay
- Vortex

## 1. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 2. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

## 3. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer LIII/CETP Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.2 **CETP Donor Molecule:**

Ready to use as supplied. Aliquot CETP donor molecule so that you have enough volume to perform the desired number of tests. Store at 4°C. Keep on ice while in use.

9.3 **Acceptor Molecule (CETP)/CETP Acceptor Molecule:**

Ready to use as supplied. Aliquot Acceptor Molecule (CETP)/CETP acceptor molecule so that you have enough volume to perform the desired number of tests. Store at 4°C. Keep on ice while in use.

9.4 **CETP Positive Control/Positive Control (Rabbit serum):**

Ready to use as supplied. Aliquot positive control so that you have enough volume to perform the desired number of tests. Store at -80°C. Keep on ice while in use.

9.5 **Torcetrapib Solution/CETP Inhibitor (Torcetrapib):**

Ready to use as supplied. Aliquot inhibitor so that you have enough volume to perform the desired number of tests. Store at 4°C. Keep on ice while in use.

## 4. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- To save time, the standard curve can be made during sample incubation.

10.1 Perform serial dilutions of the CETP Donor Molecule in 100% isopropanol as follows:

10.1.1 Dilute 10 µL of provided 8 nmol/mL Donor Molecule (Section 9.2) in 990 µL of 100% isopropanol = 80 pmol/mL CETP Donor Molecule standard.

10.1.2 Prepare a 20 pmol/mL Donor Molecule standard by adding 250 µL of standard from Section in 750 µL of 100% isopropanol = Standard #1.

10.1.3 Using Standard #1, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard # | Sample to dilute | Volume sample to dilute (µL) | Volume of 100% IPP (µL) | Final volume standard in well (µl) | Final Con in well |
|---|---|---|---|---|---|
| 1 | Step 10.1.2 | | | | 4 pmol |
| 2 | Std #1 | 500 | 500 | 200 | 2 pmol |
| 3 | Std #2 | 500 | 500 | 200 | 1 pmol |
| 4 | Std #3 | 500 | 500 | 200 | 0.5 pmol |
| 5 | Std #4 | 500 | 500 | 200 | 0.25 pmol |
| 6 | - | 0 | 500 | 200 | 0 pmol |

Each dilution has enough amount of standard to set up duplicate readings (2 x 200 µL).

Note: The Standard Curve wells should be read promptly after transferring the serially-diluted standards from microcentrifuge tubes to the 96-well plate, to minimize the evaporation of the isopropanol.

## 5. SAMPLE PREPARATION

**General Sample information:**

- Collect plasma (recommended) or serum by standard methods.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

### 11.1 Plasma or Serum:

Collect plasma or serum by standard methods.

Plasma and serum samples can be tested directly by adding sample to the microplate wells. Whenever possible, we recommend using plasma as preferred sample type.

Dilute plasma or serum 1/5 – 1/10 in Assay Buffer LIII/CETP Assay Buffer and use 2 – 10 µL to obtain signal within the range of the Standard Curve.

Using higher than recommended amounts of plasma or serum will inhibit the signal (>2 µL undiluted).

***NOTE:*** *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

### 11.2 CETP Positive Control/Positive Control (Rabbit Serum):

Dilute rabbit Serum (Section 9.4) 1/10 in Assay Buffer LIII/CETP Assay Buffer and use 10 µL of diluted positive control in the test.

### 11.3 Optional – Negative Control (Torcetrapib, 1mM):

Dilute 4 µL Torcetrapib (Section 9.5) in 496 µL DMSO. Torcetrapib will inhibit both rabbit and human CETP.

## 6. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 200 µL standard dilutions.
- Sample, background, positive and optional inhibitor wells as follows:

| Component | Sample (µL) | Background Control (µL) | Positive Control (µL) | Inhibitor ( µL) |
|---|---|---|---|---|
| **CETP Donor Molecule** | **5** | **5** | **5** | **5** |
| **Acceptor Molecule (CETP)/CETP Acceptor Molecule** | **5** | **5** | **5** | **5** |
| **Sample (Section 11.1)** | **2 – 10** | **0** | **0** | **2 – 10** |
| **Positive control (Section 11.2)** | **0** | **0** | **10** | **0** |
| **Torcetrapib Solution/CETP Inhibitor (Section 11.3)** | **0** | **0** | **0** | **2** |
| **Assay Buffer LIII/CETP Assay Buffer** | **Adjust volume to 200** | **Adjust volume to 200** | **Adjust volume to 200** | **Adjust volume to 200** |

12.2 Pre-incubate all wells (standard, samples and controls) at 37°C for 10 min protected from light to stabilize the signal.

12.3 Measure fluorescence on a microplate reader at Ex/Em = 488/523 nm in a kinetic mode, every 2 – 3 minutes, for 1 – 3 hours at 37°C.

***NOTE:*** *Sample incubation time can vary depending on CETP activity. We recommend measuring the fluorescence in kinetic mode and choosing two time points ($T_1$ and $T_2$) in the linear range to calculate the CETP activity of the samples. The*

*Standard Curve can be read in the end point mode, although we suggest to read the standard no later than 1 hour.*
*High activity samples, such as rabbit serum, may have decreased activity rate after 1 hour. If you want to run the assay for a longer period, use less sample.*

## 7. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
    - Average the duplicate reading for each standard and sample.
    - Subtract the mean absorbance value of the blank **(Standard #6)** from all standard. This is the corrected absorbance.
    - Plot the corrected absorbance values for each standard as a function of the final concentration of Donor Molecule.
    - Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
    - Subtract reagent background control reading from sample reading and extrapolate from the standard curve plotted.
    - Activity of PLTP is calculated as:

      **$\Delta RFU = (RFU_2 - RFU_{BG2}) - (RFU_1 - RFU_{BG1})$**

      Where:

      **$RFU_1$ and $RFU_2$** is the sample reading at time T1 and T2 respectively

      **$RFU_{BG1}$ and $RFU_{BG2}$** is the reagent background control reading at time T1 and T2 respectively
    - Use the ΔRFU to obtain B pmol of cholesteryl ester transferred by CETP.
    - CETP activity (in pmol/µL/hr) in the test samples is calculated as:

$$CETP\ Activity = \left(\frac{B}{\Delta T\ x\ V}\right) * D$$

= pmol/µL/hr

Where:

B = Amount of Cholesteryl Ester (Donor Molecule) from Standard Curve (pmol).

ΔT = Reaction time (hour).

V = sample volume added into the reaction well (µL).

D = sample dilution factor.

**Unit Definition:**

**1 Unit CETP activity** = amount of CETP that will transfer 1.0 µmol of Donor Molecule (Cholesteryl Ester) per hour at 37°C.

## 8. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

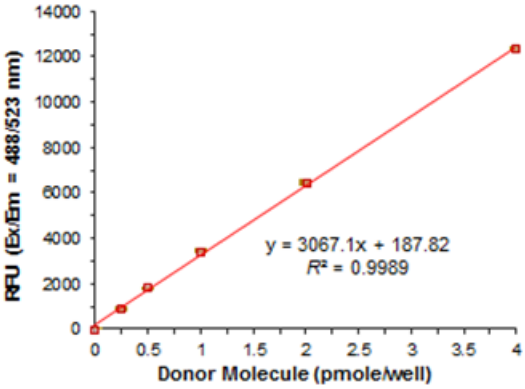



**Figure 1**. Typical Donor Molecule standard calibration curve using fluorometric reading.

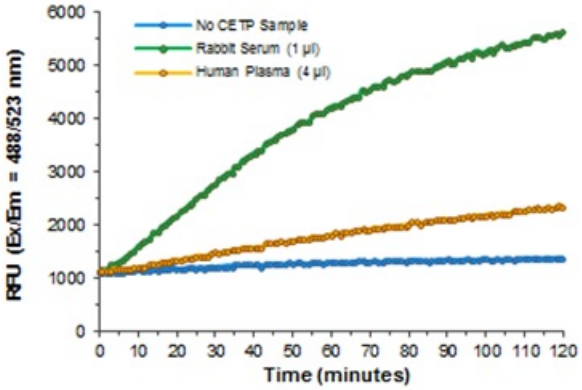



**Figure 2:** Measurement of CETP activity of rabbit serum (1 µL), and Human plasma (4 µL).

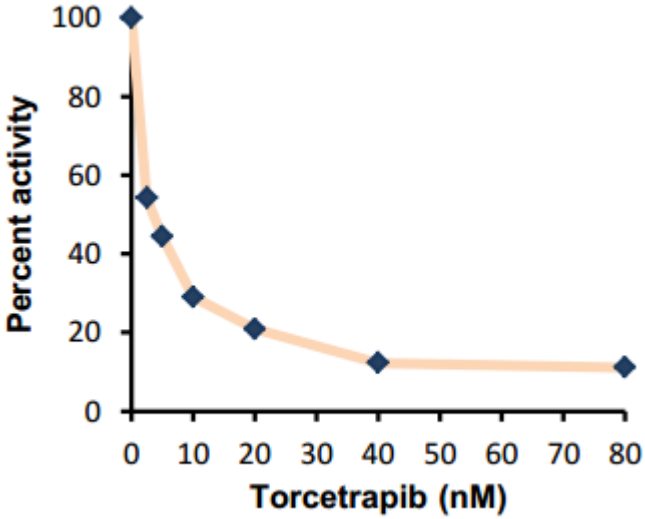



**Figure 3:** Inhibition of CETP activity from rabbit serum by Torcetrapib. The assay was run for 1 hour and the $IC_{50}$ was determined to be 3.56 nM.

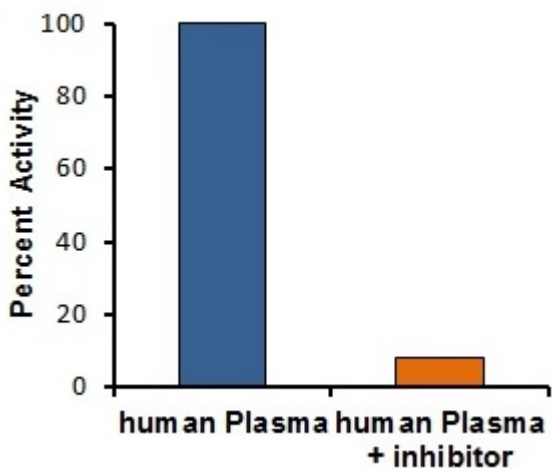



**Figure 4:** Inhibition of CETP activity from Human plasma using 80 nM Torcetrapib; assay was run for 2 hours.

## 9. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, Donor Molecule, Acceptor Molecule, inhibitor and positive control (aliquot if necessary); get equipment ready.
- Prepare standard curve using serial dilutions.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up standard reaction wells – 200 µL
- Set up rest of reaction wells as follows:

| Component | Sample (µL) | Background Control (µL) | Positive Control (µL) | Inhibitor ( µL) |
|---|---|---|---|---|
| **CETP Donor Molecule** | **5** | **5** | **5** | **5** |
| **Acceptor Molecule (CETP)/CETP Acceptor Molecule** | **5** | **5** | **5** | **5** |
| **Sample, diluted** | **2 – 10** | **0** | **0** | **2 – 10** |
| **Positive control, diluted** | **0** | **0** | **10** | **0** |
| **Torcetrapib Solution/CETP Inhibitor** | **0** | **0** | **0** | **2** |
| **Assay Buffer LIII/CETP Assay Buffer** | **Adjust volume to 200** | **Adjust volume to 200** | **Adjust volume to 200** | **Adjust volume to 200** |

- Pre-incubate 37°C for 30 min protected from light.
- Measure fluorescence on a microplate reader at Ex/Em = 488/523 nm in a kinetic mode, every 2 – 3 minutes, for 1 – 3 hours at 37°C

## 10. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| Assay not working | Use of ice-cold buffer | Buffers must be at room temperature |
| | Plate read at incorrect wavelength | Check the wavelength and filter settings of instrument |
| | Use of a different 96-well plate | Colorimetric: Clear plates<br>Fluorometric: black wells/clear bottom plate |
| Sample with erratic readings | Samples not deproteinized (if indicated on protocol) | Use PCA precipitation protocol for deproteinization |
| | Cells/tissue samples not homogenized completely | Use Dounce homogenizer, increase number of strokes |
| | Samples used after multiple free/ thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Use of old or inappropriately stored samples | Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use |
| | Presence of interfering substance in the sample | Check protocol for interfering substances; deproteinize samples |
| Lower/ Higher readings in samples and Standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Allowing reagents to sit for extended times on ice | Always thaw and prepare fresh reaction mix before use |
| | Incorrect incubation times or temperatures | Verify correct incubation times and temperatures in protocol |

| Problem | Cause | Solution |
|---|---|---|
| Standard readings do not follow a linear pattern | Pipetting errors in standard or reaction mix | Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the tubes |
| | Standard stock is at incorrect concentration | Always refer to dilutions on protocol |
| Unanticipated results | Measured at incorrect wavelength | Check equipment and filter setting |
| | Samples contain interfering substances | Troubleshoot if it interferes with the kit |
| | Sample readings above/ below the linear range | Concentrate/ Dilute sample so it is within the linear range |

## 11. FAQ

## 12. INTERFERENCES

## 13. NOTES

## Technical Support



**For all technical or commercial enquiries please go to:**
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)