DRG® CETP Activity Assay Kit (EIA-4414)

Revised 27 May 2011 rm (Vers. 2.1) **RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 KIT CONTENTS

100 assays; Store at 4°C

Component	Volume	Cap color
Donor Molecule	1 ml	Green
Acceptor Molecule	1 ml	Blue
CETP Assay Buffer (10X)	5 ml	Clear
Positive Control (Rabbit Serum)	30 µl	Red

2 CETP ASSAY PROTOCOL

2.1 General Consideration for Using Fluorometer and Plate Reader

We recommend using a microtiter plate for the assay. The microtiter plates should be sealed as tightly as possible with plate sealer and incubated in a sealed, humidified chamber to prevent evaporation.

If using a regular fluorometer for sample reading, the samples should be diluted to 500 µl with 1X CETP Assay Buffer before read.

2.2 Preparation of Standard Curve

Standard curve is prepared by making serial dilutions of the donor molecule in isopropanol and subsequently recording the fluorescence intensity of each dilution, using isopropanol alone as a blank.

1. Prepare 6 test tubes labeled T0 to T5, each contains 0.2 ml of isopropanol; the tube labeled T5 should contain an additional 0.2 ml of isopropanol.
2. Add 2 µl Donor Molecule to T5, vortex to mix well.
3. Transfer 0.2 ml from T5 to T4. Mix and then transfer 0.2 ml from T4 to T3. Mix and then transfer 0.2 ml from T3 to T2. Mix and then transfer 0.2 ml from T2 to T1. The Donor Molecule solution contains 0.1 mM labeled lipids and thus the standard curve samples contain 0, 6.25, 12.5, 25, 50, 100 pmol donor molecule.
4. Read the fluorescence intensity (Ex. = 465 nm; Em. = 535 nm) of the samples from T0 to T5.
5. Apply the fluorescence intensity values of the standard curve directly to your results to express activity of the plasma sample (pmoles/ml plasma/hr), or use Relative Fluorescence Units (RFU)/µl plasma/hr.

2.3 Assay Procedure

1. For each reaction, add the following components:

10 µl	Donor Molecule
10 µl	Acceptor Molecule
20 µl	10X CETP Assay Buffer
1-3 µl	Your Sample (serum or plasma)
ddH_2O	To a total of 200 µl

   For positive control, add 1-3 µl of Rabbit Serum instead of your sample.

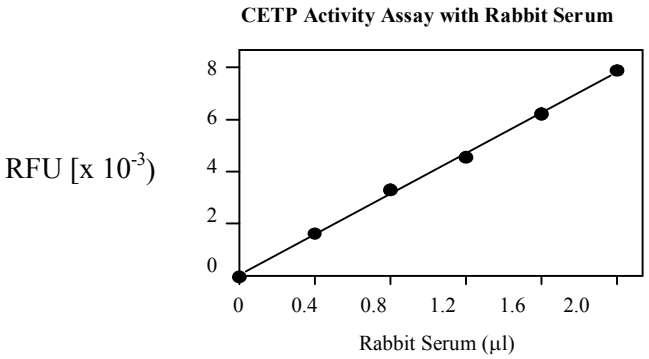
   Prepare a blank that contains no CETP Source.

2. Incubate for 30-60 minutes at 37°C.
3. Measure the fluorescence intensity of the blank, samples, and positive control using a fluorescence plate reader or fluorometer (Ex. = 465 nm; Em. = 535 nm). Due to the nature of the self-quenched probe, background fluorescence can be significant; therefore, fluorescence intensity from each sample should be corrected by subtracting the blank fluorescence intensity. The increase in fluorescence intensity is usually 0.2-2 fold over blank.
4. Calculate the activity of the plasma sample:

   $Y = MX + B$

   Where: Y = Fluorescence Intensity of Sample – Fluorescence Intensity of Blank
   M = Slope of the Standard Curve
   X = Concentration of Plasma Sample
   B = Intercept

   Example: Y = 16283 – 8655 = 7628
   M = 74.5
   B = 480
   7628 = 74.5X + 480
   X = 95.9 pmol/µl sample/1 hr



3 GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	Use of ice-cold assay buffer	Assay buffer must be at room temperature
	Omission of a step in the protocol	Refer and follow the data sheet precisely
	Plate read at incorrect wavelength	Check the wavelength in the data sheet and the filter settings of the instrument
	Use of a different 96-well plate	Fluorescence: Black plates (clear bottoms) Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	Use of an incompatible sample type	Refer data sheet for details about incompatible samples
	Samples prepared in a different buffer	Use the assay buffer provided in the kit or refer data sheet for instructions
	Cell/ tissue samples were not completely homogenized	Use Dounce homogenizer (increase the number of strokes) observe for lysis under microscope
	Samples used after multiple free-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	Troubleshoot if needed
	Use of old or inappropriately stored samples	Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Refer datasheet & verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in the standard	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Prepare a master reaction mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at an incorrect concentration	Always refer the dilutions in the data sheet
	Calculation errors	Recheck calculations after referring the data sheet
	Substituting reagents from older kits/ lots	Use fresh components from the same kit
Unanticipated results	Measured at incorrect wavelength	Check the equipment and the filter setting

	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Use of incompatible sample type	Refer data sheet to check if sample is compatible with the kit or optimization is needed
	Sample readings above/below the linear range	Concentrate/ Dilute sample so as to be in the linear range

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