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Carboxylesterase Activity Assay Kit (Fluorometric)

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(Catalog # K2014-100, 100 assays; Store at -20°C)

I. Introduction:

Human Carboxylesterase 1 (E.C. 3.1.1.1, CES1, hCE1, CES1b or CES1A1) is a α,β-serine hydrolase expressed in most tissues with higher levels in the liver. It plays a crucial role in the metabolism of various ester xenobiotics including many ester drugs (such as oseltamivir, clopidogrel, irinotecan and capecitabine) and environmental toxicants (such as pyrethroids). Additionally, Carboxylesterases are known to metabolize endogenous esters including triacylglycerols, cholesteryl esters, and other endogenous lipids thereby maintaining lipid homeostasis. More specifically, Carboxylesterases catalyze the ester cleavage of a large number of diverse ester- or amide-containing substrates into alcohol and carboxylic acid. **BioVision's Carboxylesterase Activity Assay Kit** is a simple, rapid, plate-based fluorometric assay for measuring Carboxylesterase activity in biological samples. The kit uses the proprietary substrate for the quantification of Carboxylesterase (CE) activity in samples. The assay can detect as low as 6.6 µU of Carboxylesterase activity.

Carboxylesterase Substrate $\xrightarrow{\text{CE}}$ **Carboxylesterase Product (Fluorescent Product, Ex/Em = 490/550 nm)**

II. Applications:

Carboxylesterase activity in cell or tissue lysates

III. Sample Type:

- Mammalian Tissues (i.e. rat and human liver microsomes, S9 fraction)
- Cell Culture (i.e. HepG2 cell lysate)

IV. Kit Contents:

Components	K2014-100	Cap Code	Part Number
CE Assay Buffer	13 ml	NM	K2014-100-1
CE Substrate	50 µl	Blue	K2014-100-2
CE Standard	50 µl	Yellow	K2014-100-3
CE Positive Control	1 vial	Green	K2014-100-4

V. User Supplied Reagents and Equipment:

- dH_2O
- 1X PBS
- Black 96-well microplate with flat bottom
- Multi-well spectrophotometer (plate reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay. Allow the kit contents to thaw at room temperature (RT).

- **CE Assay Buffer:** Store at -20°C. Immediately before use, bring to RT.
- **CE Substrate:** Thaw and aliquot into amber vials. Protect from light and store at -20°C.
- **CE Standard (10 mM):** Thaw at RT. Aliquot into separate vials and store at -20°C for long term storage.
- **CE Positive Control:** Reconstitute the vial with 20 µl of dH_2O. Aliquot the reconstituted Positive Control into vials and store at -80°C for up to one year. Avoid repeated freeze thaw cycles.

VII. Carboxylesterase Activity Assay Protocol:

1. Sample Preparation: Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl CE Assay buffer to perform lysis. Keep on ice for 10 min followed by centrifugation at 10,000 x *g* and 4ºC for 15 min. Collect the supernatant. Prepare several dilutions of the supernatant. Keep diluted supernatant on ice. Add 2-50 µl of each supernatant dilution (in duplicates) to wells of a 96-well black plate labeled as "**Sample**" and "**Sample Background**" respectively. Adjust the volume to 50 µl/well with CE Assay Buffer. Add 50 µl of CE Assay Buffer to a "**Reagent Background**" well and 100 µl of CE Assay Buffer to a "**Blank**" well respectively.

CE Positive Control: Add 2 µl of the reconstituted CE Positive Control to 498 µl of CE Assay Buffer to prepare a CE Positive Control Working Solution. Add 50 µl of the CE Positive Control working solution into a well labeled as "**Positive Control**".

Notes:

a. For Unknown Samples, we suggest doing a pilot experiment and testing several doses to ensure the RFU readings are within the range of the CE Standard Curve.
b. For Samples having background RFU values, prepare parallel Sample well(s) labeled as Sample Background.

2. Standard Curve Generation: Dilute 10 mM stock CE Standard to 1 mM CE Standard solution by adding 5 µl of 10 mM Standard to 45 µl of dH_2O. Dilute 1 mM CE Standard solution further to 50 µM CE Standard solution by adding 10 µl of 1 mM CE Standard to 190 µl of dH_2O. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM CE Standard into a series of wells of a 96-well plate to generate 0, 100, 200, 300, 400, 500 pmoles/well CE Standard. Bring the volume of each Standard well to 100 µl with CE Assay Buffer.

3. CE Reaction Mix Preparation: Prepare enough reagents for the number of assays to be performed. Make sufficient amounts of the CE Reaction Mix to add 50 µl to all assay wells. Dilute the stock CE Substrate 10 fold by adding 6 µl of the stock CE Substrate solution to 54 µl of dH_2O. Add further 940 µl of dH_2O to the diluted substrate to prepare the CE Reaction Mix. Add 50 µl of the CE Reaction Mix to Positive Control, Reagent Background and Sample wells.

Positive Control: 50 µl CE Positive Control and 50 µl CE Reaction Mix
Reagent Background: 50 µl CE Assay Buffer and 50 µl CE Reaction Mix
Sample: 50 µl Sample and 50 µl CE Reaction Mix
Sample Background: 50 µl Sample and 50 µl CE Assay Buffer
Blank: 100 µl CE Assay Buffer

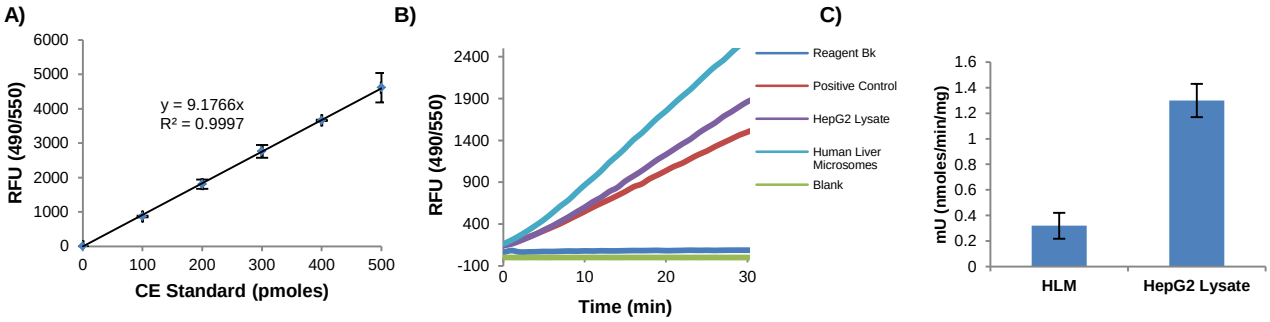
4. Measurement: Read the fluorescence in kinetic mode every min interval for 1 hr at Ex/Em = 490/550 nm. The CE Standard Curve can be read in endpoint mode (i.e. at the end of the incubation time).

5. Calculation: Subtract 0 Standard RFU values from all Standard readings. Plot the CE Standard Curve. If the Sample Background reading is significant, subtract the Sample Background reading from its paired Sample readings. Choose any two time points within the linear portion of the curve (t_1 & t_2) for each Sample. Apply the corrected Sample RFU values to the CE Standard Curve to get B pmol of CE generated during the reaction time ($\Delta t = t_2 - t_1$). Calculate the Sample Carboxylesterase activity as shown below:

Sample Carboxylesterase Activity = B/(Δt*V) X D pmol/ml or µU/ml

Where B is the amount of CE in the Sample well (pmol)
Δt (min)
V is the Sample volume added into the reaction well (ml)
D is the Sample dilution factor

Unit Definition: One Unit of Carboxylesterase Activity is the amount of enzyme that generates 1 µmole of product per minute at 37°C.



Figures: A). CE Standard Curve (0-500 pmoles/well). **B).** CE activity in HepG2 Lysate, Human Liver Microsomes and Positive Control **C).** CE Activity in Human Liver Microsomes (HLM, 16 mg/ml), and HepG2 Lysate (3.21 mg/ml). Assay was performed following the kit protocol.

VIII. Related Products:

Fatty Acid Amide Hydrolase (FAAH) Activity Assay Kit (Fluorometric) (K434)
Soluble Epoxide Hydrolase Activity Assay Kit (Fluorometric) (K477)
Soluble Epoxide Hydrolase Inhibitor Screening Kit (Fluorometric) (K480)
Butyrylcholinesterase Activity Kit (Colorimetric) (K516)
Human Enolase α Inhibitor Screening Kit (Colorimetric) (K526)
Enolase Activity Colorimetric/Fluorometric Assay Kit (K691)

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