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ab273314 Carboxylesterase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273314
(use https://www.abcam.cn/ab273314 for china, or
https://www.abcam.co.jp/ab273314 for Japan)

For the determination of Carboxylesterase activity in cell and tissue lysates.

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.

Table of Contents

1. Overview

Carboxylesterase Activity Assay Kit (Fluorometric) (ab273314) 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.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix to the appropriate wells.

Measure fluorescence in kinetic mode every minute for 1 hour.

Determine Carboxylesterase activity using equation.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
CES Assay Buffer/CE Assay Buffer	13 mL	-20°C
CES1 Substrate/CE Substrate	50 µL	-20°C
CES1 Fluorescence Standard/CE Standard	50 µL	-20°C
CES1 Positive Control/CE Positive Control (lyophilized)	1 vial	-20°C

7. Materials Required, Not Supplied

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

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

8. 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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening. Allow the kit contents to thaw at room temperature (RT).

9.1 **CES Assay Buffer/CE Assay Buffer:**
Store at -20 °C. Bring to RT immediately before use.

9.2 **CES1 Substrate/CE Substrate:**
Thaw and aliquot into amber vials. Protect from light and store at -20°C.

9.3 **CES1 Fluorescence Standard/CE Standard:**
Thaw at RT. Aliquot into separate vials and store at -20°C for long term storage.

9.4 **CES1 Positive Control/CE Positive Control (Lyophilized):**
Reconstitute the vial with 20 µl of dH_2O. Aliquot the reconstituted CES1 Positive Control/Positive Control into vials and store at -80°C for
up to one year. Avoid repeated freeze thaw cycles.

10. Sample Preparation

10.1 Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl CES Assay Buffer/CE Assay buffer to perform lysis.

10.2 Keep on ice for 10 mins followed by centrifugation at 10,000 *x g* and 4°C for 15 mins. Collect the supernatant.

10.3 Prepare several dilutions of the supernatant. Keep diluted supernatant on ice.

10.4 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.

10.5 Adjust the volume to 50 µl/well with CES Assay Buffer/CE Assay Buffer.

10.6 Add 50 µl of CES Assay Buffer/CE Assay Buffer to a Reagent Background well and 100 µl of CES Assay Buffer/CE Assay Buffer to a Blank well respectively.

10.7 **CES1 Positive Control/CE Positive Control**: Add 2 µl of the reconstituted CES1 Positive Control/CE Positive Control to 498 µl of CES Assay Buffer/CE Assay Buffer to prepare a CES1 Positive Control/CE Positive Control Working Solution.

10.8 Add 50 µl of the CES1 Positive Control/CE Positive Control working solution into a well labeled as Positive Control.

Δ Note: 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.

Δ Note: For Samples having background RFU values, prepare parallel Sample wells labeled as Sample Background.

11. Standard Curve

11.1 Dilute 10 mM stock CES1 Fluorescence Standard/CE Standard to 1 mM CES1 Fluorescence Standard/CE Standard solution by adding 5 µl of 10 mM Standard to 45 µl of dH_2O.

11.2 Dilute 1 mM CES1 Fluorescence Standard/CE Standard solution further to 50 µM CES1 Fluorescence Standard/CE Standard solution by adding 10 µl of 1 mM CES1 Fluorescence Standard/CE Standard to 190 µl of dH_2O.

11.3 Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM CES1 Fluorescence Standard/CE Standard into a series of wells of a 96-well plate to generate 0, 100, 200, 300, 400, 500 pmol/well CES1 Fluorescence Standard/CE Standard.

11.4 Bring the volume of each Standard well to 100 µl with CES Assay Buffer/CE Assay Buffer.

Standard #	50 µM CE-Standard (µL)	CES Assay Buffer/CE Assay Buffer (µL)	CE (pmol/well)
1	10	90	500
2	8	92	400
3	6	94	300
4	4	96	200
5	2	98	100
6	0	100	0

12. Assay Procedure

Reaction mix:

12.1 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.

12.2 Dilute the stock CES1 Substrate/CE Substrate 10-fold by adding 6 µl of the stock CES1 Substrate/CE Substrate solution to 54 µl of dH_2O.

12.3 Add further 940 µl of dH_2O to the diluted CES1 Substrate/substrate to prepare the CE Reaction Mix.

Positive Control: 50 µl CES1 Positive Control/CE Positive Control and 50 µl CE Reaction Mix **Reagent Background**: 50 µl CES Assay Buffer/CE Assay Buffer, 50 µl CE Reaction Mix
Sample: 50 µl Sample and 50 µl CE Reaction Mix Sample
Background: 50 µl Sample and 50 µl CES Assay Buffer/CE Assay Buffer
Blank: 100 µl CES Assay Buffer/CE Assay Buffer

12.4 Add 50 µl of the CE Reaction Mix to CES1 Positive Control/Positive Control, Reagent Background and Sample wells.

12.5 Read the fluorescence in kinetic mode every minute for 1 hour at Ex/Em = 490/550 nm.

Δ Note: The CE Standard Curve can be read in endpoint mode (i.e. at the end of the incubation time).

13. Calculations

13.1 Subtract 0 Standard RFU values from all Standard readings. Plot the CE Standard Curve.

13.2 If the Sample Background reading is significant, subtract the Sample Background reading from its paired Sample readings.

13.3 Choose any two time points within the linear portion of the curve (t1 and t2) for each Sample.

13.4 Apply the corrected Sample RFU values to the CE Standard Curve to get B pmol of CE generated during the reaction time ($\Delta t = t2-t1$).

13.5 Calculate the Sample Carboxylesterase activity as shown below:

$$Sample\ CE\ activity\ = \frac{B}{(\Delta t\ x\ V)}\ \times D\ (pmol/ml)$$ or µU/mL

B= Amount of CE in the Sample well (pmol)
Δt (min)
V= Sample volume added into the reaction well (ml)
D= 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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

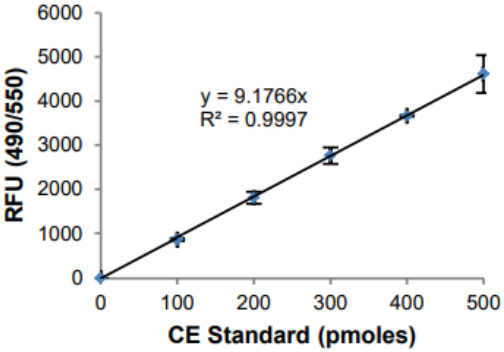

Figure 1. Carboxylesterase Standard Curve (0-500 pmoles/well).

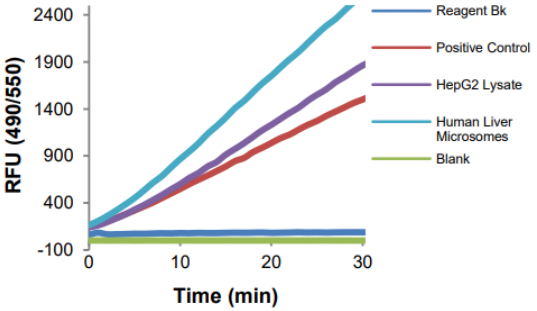

Figure 2. Carboxylesterase activity in HepG2 lysate, human liver microsomes and Positive Control.

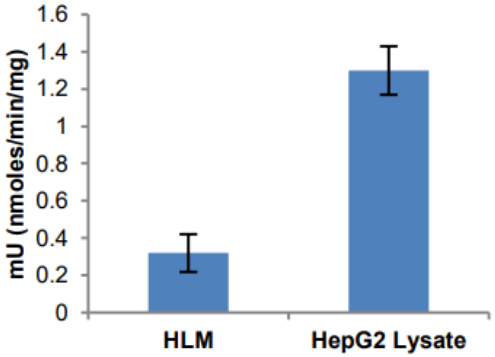

Figure 3. Carboxylesterase activity in human liver microsomes (HLM) (16 mg/ml), and HepG2 lysate (3.21 mg/ml).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)