CarboxylesteraseMicroplate Assay Kit

User Manual

Catalog # CAK1227

(Version 1.2A)

Detection and Quantification of CarboxylesteraseActivity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

A carboxylesterase or carboxylic-ester hydrolase is an enzyme that catalyzes a chemical reaction of the form a carboxylic ester + H_2O ⇌ an alcohol + a carboxylate Thus, the two substrates of this enzyme are carboxylic ester and H_2O, whereas its two products are alcohol and carboxylate. Most enzymes from this group are serine hydrolases belonging to the superfamily of proteins with alpha/beta hydrolase fold.Carboxylesterases have a wide tissue distribution and are found in the greatest amounts in the liver and in the gastrointestinal tract, brain, and possibly blood. Carboxylesterases belongs to the class of serine hydrolases that include acetylcholinesterase, which is primarily found in the blood and neural synapses. Carboxylesterase Microplate Assay Kit is based on hydrolysis of substrate to α-Naphthol. The intensity of the product color, measured at 600 nm, is proportional to the Carboxylesteraseactivity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Diluent	1 mlx 2	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate:add 1 ml Diluent to dissolve before use.

Dye Reagent: add 10 ml Distilled water to dissolve before use.

Standard: add 1 ml Diluent to dissolve, then add 4µl into 996µldistilled water, mix; the concentration will be 200µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank
Sample	20µl	--	--	--
Standard	--	--	100µl	--
Assay Buffer	70µl	90µl	--	100µl
Substrate	10 µl	10 µl	--	--
Mix, put it in the oven,37 °C for 10 minutes.				
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix,wait for 10 minutes, record absorbance measured at 600 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Carboxylesterase activity is defined asthe enzyme generates 1 μmolα-Naphthol per minute.

1. According to the protein concentration of sample

$$\text{Carboxylesterase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T$$

$$= 0.1 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Carboxylesterase (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.1 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$\text{Carboxylesterase (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.1 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

W: theweight of sample, g;

$C_{Standard}$: the concentration of standard, 200μmol/L = 0.2μmol/ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

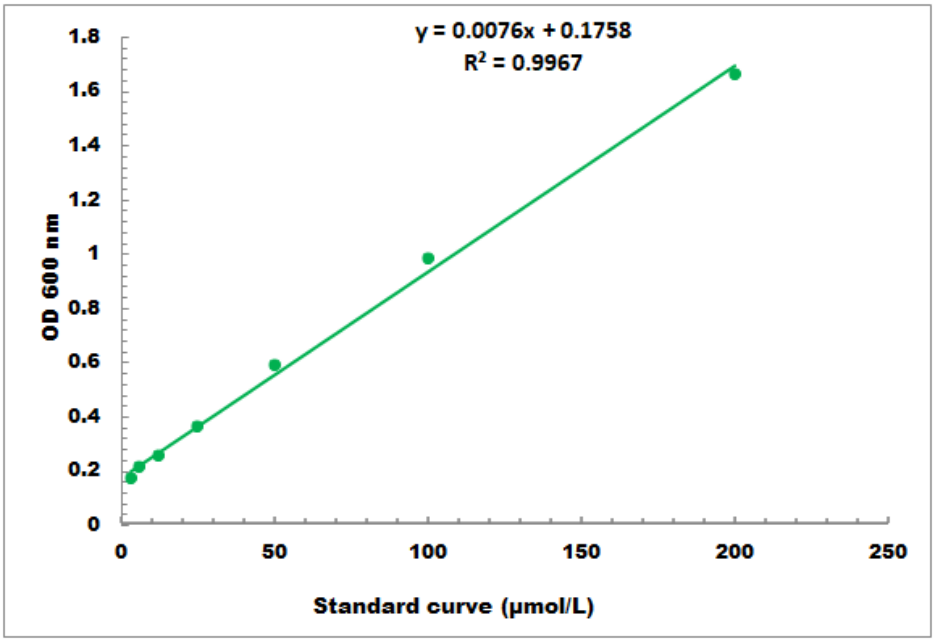
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2μmol/L -200μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES