# CarboxylesteraseActivity

# Colorimetric Microplate Assay Kit

# User Manual

**Catalog # CAK1227**

(Version 1.3A)

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. CarboxylesteraseActivity Colorimetric 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 | |
| Technical Manual | 1 Manual | |

## 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. REAGENT PREPARATION

**Standard**:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 50 mmol/L of standardstock solution.Keep in dark and store at 4 °C for 2-3 days or -20°C for 1 month after reconstitution.Then dilute to 0.2 mmol/Lstandard top solutionby adding 4 µlstock solution into 996 µldistilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.12µmol/L.

**Substrate:**Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use.Keep in dark and store at 4 °C for 1 months or -20°C for 4-5months after reconstitution.

**Dye Reagent**: Add 10 ml Distilled water to dissolve before use.Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

**Note:** Divide into small aliquots to avoid repeated freeze-thaw cycles.

## V. 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 $5\times10^6$ 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.

## VI. 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:**

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

## VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

2.4According to the quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times N\times (V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, mL

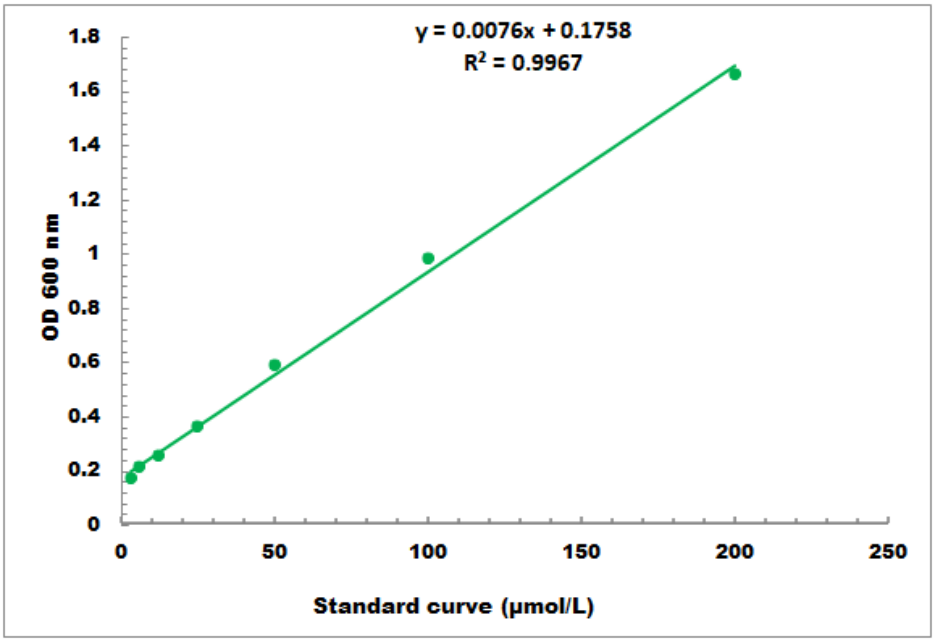
$V_{Sample}$: the volume of sample in assay procedure, mL

$V_{Assay}$: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

## VIII. TYPICAL DATA

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




Detection Range: 2μmol/L -200μmol/L