

APC374Hu61 100µg

Active Carboxylesterase 1 (CES1)

Organism Species: *Homo sapiens (Human)*

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Gly18~Leu567

Tags: N-terminal His-tag

Purity: >95%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 10mM PBS, pH7.6, containing 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.5

Predicted Molecular Mass: 61.6kDa

Accurate Molecular Mass: 62kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were

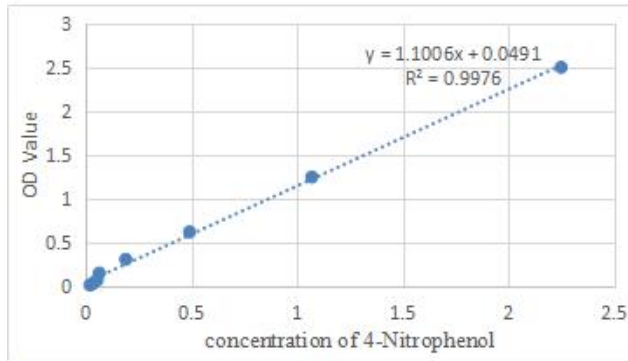
observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[**SEQUENCE**]

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          GHP  SSPPVVDTVH  GKVLGKFVSL  EGFAQPVAIF
LGIPFAKPPL  GPLRFTPPQP  AEPWSFVKNA  TSYPMCTQD  PKAGQLLSEL
FTNRKENIPL  KLSDECLYLN  IYTPADLTKK  NRLPVMVWIH  GGGLMVGAAS
TYDGLALAAH  ENVVVVTIQY  RLGIWGFFST  GDEHSRGNWG  HLDQVAALRW
VQDNIAFSGG  NPGSVTIFGE  SAGGESVSVL  VLSPLAKNLF  HRAISESGVA
LTSVLVKKGD  VKPLAEQIAI  TAGCKTTTSA  VMVHCLRQKT  EEELLETTLK
MKFLSLDLQG  DPRESQPLLG  TVIDGMLLLK  TPEELQAERN  FHTVPYMGVI
NKQEFGLWIP  MQLMSYPLSE  GQLDQKTAMS  LLWKSYPVLC  IAKELIPEAT
EKYLGGTDDT  VKKKDLFLDL  IADVMFGVPS  VIVARNHRDA  GAPTYMYEFQ
YRPSFSSDMK  PKTVIGDHGD  ELFSVFGAPF  LKEGASEEEI  RLSKMVMKFW
ANFARNGNPN  GEGLPHWPEY  NQKEGYLQIG  ANTQAAQKLK  DKEVAFWTNL
FAKKAWEKPP  QTEHIEL
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[**ACTIVITY**]

carboxylesterase 1 (CES1) also known as Liver carboxylesterase 1 is a serine esterase and member of a large multigene carboxylesterase family. The protein involved in the detoxification of xenobiotics and in the activation of ester and amide prodrugs. Hydrolyzes aromatic and aliphatic esters, but has no catalytic activity toward amides or a fatty acyl-CoA ester. Hydrolyzes the methyl ester group of cocaine to form benzoylecgonine. Thus, the recombinant human CES1 activity was measured by its ability to hydrolyze 4-Nitrophenyl acetate (4-NPA) to 4-Nitrophenol. The reaction was performed in 50 mM Tris, pH 7.5 (Assay Buffer), initiated by addition 50 μ L of various concentrations of CES1 (dilute by Assay Buffer) to 50 μ L of 2 mM Substrate 4-NPA (100 mM stock in Acetone, dilute by deionized water). Incubated at 37°C for 10min, then read at a wavelength of 400 nm.



4-Nitrophenol (product)mM	OD400nm
2.5	2.248
1.25	1.069
0.625	0.4905
0.3125	0.189
0.15625	0.064
0.078125	0.0535
0.0390625	0.0345
0.01953125	0.02

Figure 1. The standard curve of 4-Nitrophenol

One unit of enzyme activity is defined as the 1µg of enzyme required to convert 1pmol of 4-Nitrophenyl acetate to 4-Nitrophenol in 1min at 37°C. The specific activity of recombinant human CES1 is 48166 pmol/min/µg.

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\Delta OD * F}{T * N}$$

ΔOD=Adjusted for Substrate Blank

F=Conversion Factor(convert from standard curve of 4-Nitrophenol)

T= Time

N=Amount of enzyme

[IDENTIFICATION]

38AATTGGGCGATCCGCTCTGGCCACCTGTGGTGGACACCGCTGCAATGCAAGTGCCTGGGGAMGTTCTGTCAGCTTAGAAGGATTGTGACAGCCTGTGGGCATTTTCTGGGAAATCCCTTTTGGCAAGCCGCTCTTGGAGCCCTGAGGTTTACTCCACCGCAGCCTGCAGAACCATGG

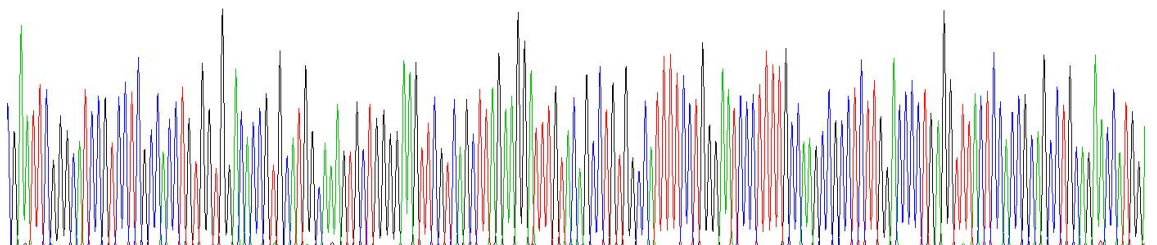


Figure 2. Gene Sequencing (extract)

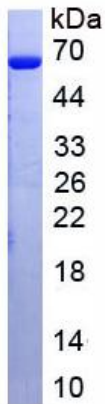


Figure 3. SDS-PAGE

Sample: Active recombinant CES1, Human

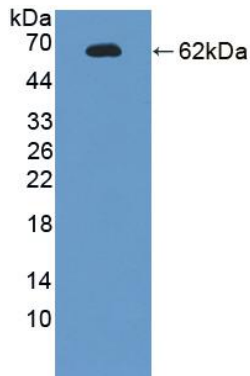


Figure 4. Western Blot

Sample: Recombinant CES1, Human;

Antibody: Rabbit Anti- Human CES1 Ab (PAC374Hu06)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.