APC374Mu01 50μg
Active Carboxylesterase 1 (CES1)
Organism Species: *Mus musculus (Mouse)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: His19~Leu565
Tags: N-terminal His-tag
Purity: >80%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Original Concentration: 50μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.9
Predicted Molecular Mass: 64.5kDa
Accurate Molecular Mass: 65/30kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1.Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in ddH_2O to a concentration of 0.1-0.2 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8^oC for one month.

Aliquot and store at -80^oC 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^oC 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.

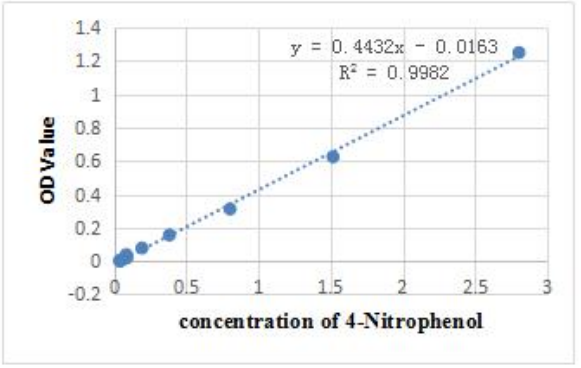
[SEQUENCE]

HP SLPPVVHTVH GKVLGKYVTL EGFSQPVAVF
LGVPFAKPPL GSLRFAPPEP AEPWSFVKHT TSYPPLCYQN PEAALRLAEL
FTNQRKIIPH KFSEDCLYLN IYTPADLTQN SRLPVMVWIH GGGLVIDGAS
TYDGVPLAVH ENVVVVVIQY RLGIWGFFST EDEHSRGNWG HLDQVAALHW
VQDNIANFGG NPGSVTIFGE SAGGESVSVL VLSPLAKNLF HRAIAQSSVI
FNPCLFGRAA RPLAKKIAAL AGCKTTTSAA MVHCLRQKTE DELLEVSLKM
KFGTVDFLGD PRESYPFLPT VIDGVLLPKA PEEILAEKSF NTVPYMVGIN
KHEFGWIIPM FLDFPLSERK LDQKTAASIL WQAYPILNIS EKLIPAAIEK
YLGGTEDPAT MTDLFLDLIG DIMFGVPSVI VSRSHRDAGA PTYMYEYQYR
PSFVSDDRPQ ELLGDHADEL FSVWGAPFLK EGASEEEINL SKMVMKFWAN
FARNGNPNGE GLPHWPEYDQ KEGYLQIGVP AQAAHRLKDK EVDFWTELRA
KETAERSSHR EHVEL

[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 mouse 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, 150 mM NaCl, pH 7.5(Assay Buffer), ainitiated by addition 50 µL of various concentrations of CES1(dilute by Assay Buffer) to 50 µL of 2 mM Substrate 4-NPA(100 mM stock in Acetone, dilute by deionized water). Incubated at 37℃ for 10min, then read at a wavelength of 400 nm.



4-Nitrophenol (product)mM/L	OD400nm
1.25	2.806
0.625	1.5165
0.3125	0.8025
0.15625	0.382
0.078125	0.1915
0.0390625	0.0825
0.01953125	0.086
0.009765625	0.0545
0.004882813	0.044
0.002441406	0.036
0.001220703	0.0405

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 mouse CES1 is 158 pmol/min/µg.

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

△OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

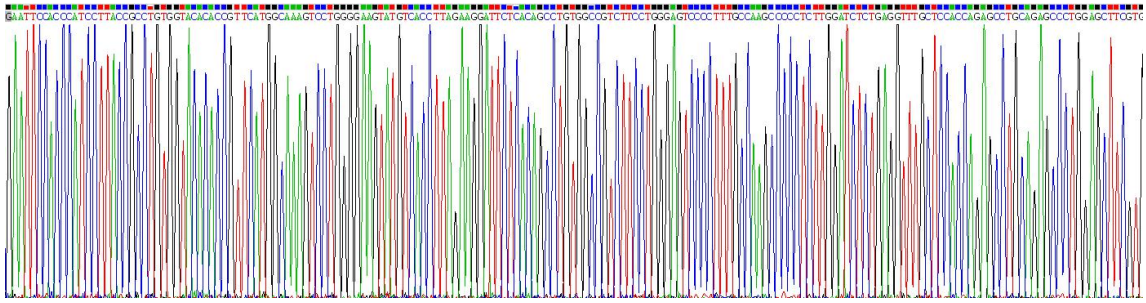


Figure 2. Gene Sequencing (extract)

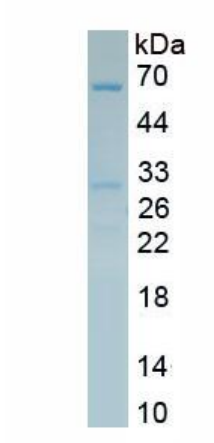


Figure 3. SDS-PAGE

Sample: Active recombinant CES1, Mouse

[IMPORTANT NOTE]

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