APR624Hu01 100μg
Active Carboxylesterase 5A (CES5A)
Organism Species: *Homo sapiens (Human)*
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: His383~Phe570
Tags: N-terminal His and GST Tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 5.9
Predicted Molecular Mass: 51.7kDa
Accurate Molecular Mass: 54kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) 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.

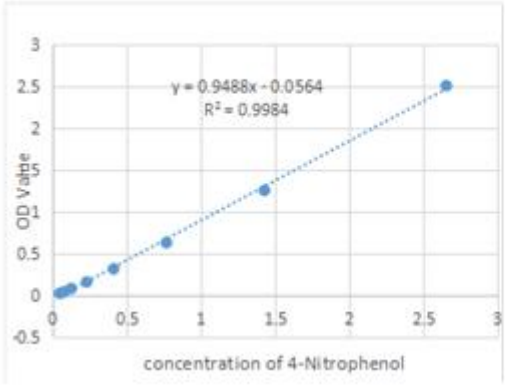
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

HLVANEYFHDKHSLTEIRDSLLDLLGDVFFVVPALITARYHRDAGAPVYFYEFRHRPQCFEDTKPAFVKADHADEVRFVFGGAFLKGD
IVMFEGATEEEKLLSRKMMKYWATFARTGNPNGNDLSLWPAYNLTEQYLQLDLNMSLGQRLKEPRVDFWTSTIPLILSASDMLHSPLS
SLTFLSLLQPFF

[ACTIVITY]

carboxylesterase 5A (CES5A) is a serine esterase and a 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 CES5A 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 μL of various concentrations of CES5A (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	OD400nm
0.01953125	0.045
0.0390625	0.076
0.078125	0.123
0.15625	0.227
0.3125	0.409
0.625	0.766
1.25	1.426
2.5	2.653

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 CES5A is 1300 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]

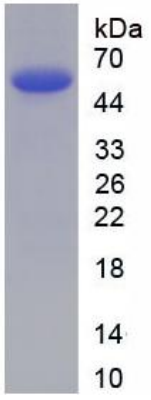


Figure 2. SDS-PAGE

Sample: Active recombinant CES5A, Human

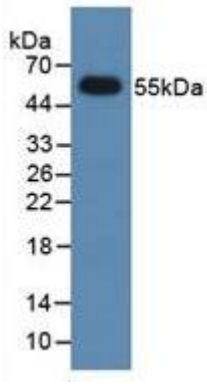


Figure 3. Western Blot

Sample: Recombinant CES5A, Human;

Antibody: Rabbit Anti- Human CES5A Ab (PAR624Hu01)

[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.