APD072Hu01 100μg
Active Carbonic Anhydrase VB, Mitochondrial (CA5B)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Cys34~Pro317
Tags: N-terminal His-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 .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 6.6
Predicted Molecular Mass: 36.3kDa
Accurate Molecular Mass: 36kDa 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 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

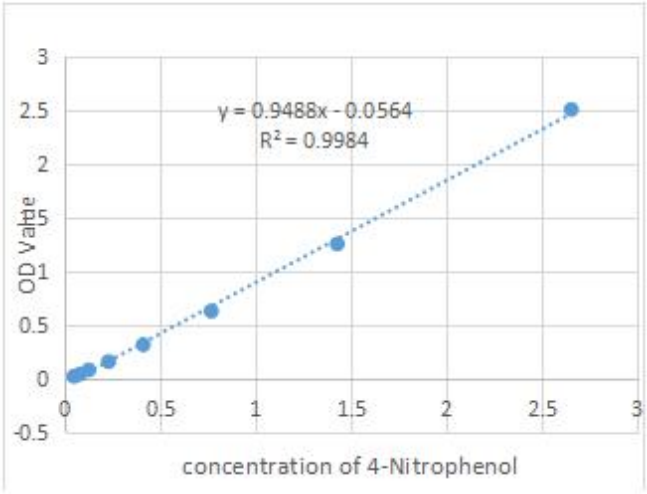
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[SEQUENCE]

CSLYTCT YKTRNRALHP LWESVDLVPG
GDRQSPINIR WRDSVYDPGL KPLTISYDPA TCLHVWNNGY SFLVEFEDST DKSVIKGGPL
EHNYRLKQFH FHWGAIDAWG SEHTVDSKCF PAELHLVHWN AVRFENFEDA ALEENGLAVI
GVFLKLGKHH KELQKLVDTL PSIKHKDALV EFGSFDPSCL MPTCPDYWTY SGSLTTPPLS
ESVTWIIKKQ PVEVDHDQLE QFRTLLFTSE GEKEKRMVDN FRPLQPLMNR TVRSSFRHDY
VLNVQAKPKP ATSQATP

[ACTIVITY]

Carbonic Anhydrase (CA) catalyzes the reversible reaction of CO2 + H2O = HCO3- + H+, which is fundamental to many processes such as respiration, renal tubular acidification and bone resorption. Carbonic Anhydrase VB encoded by the CA5B gene is a mitochondrial protein. In comparison with another mitochondrial CA (CA5A), CA5B has different tissue distribution and chromosomal location. The activity of recombinant human CA5B was measured by its ability to hydrolyze 4-Nitrophenyl acetate (4-NPA) to 4-Nitrophenol. The reaction was performed in 12.5 mM Tris, 75 mM NaCl, pH 7.5 (assay buffer), initiated by addition 50 μL of various concentrations of CA5B (diluted by assay buffer) to 50 μL of 2 mM substrate 4-NPA (100 mM stock in Acetone, diluted by assay buffer). Incubated at 37℃ for 5min, 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 1 μg of enzyme required to convert 1 pmol of 4-Nitrophenyl acetate to 4-Nitrophenol in 1min at 37°C. The specific activity of recombinant human CA5B is > 50 pmol/min/μg.

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]

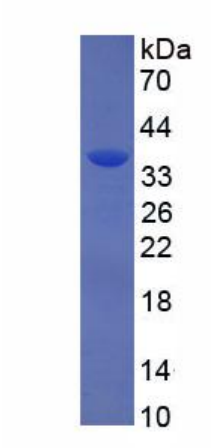


Figure 2. SDS-PAGE

Sample: Active recombinant CA5B, Human

[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.