APD076Hu61 100μg
Active Carbonic Anhydrase IX (CA9)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Pro59~Asp414
Tags: N-terminal His-tag
Purity: >90%
Traits: Freeze-dried powder
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 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: 4.2
Predicted Molecular Mass: 40.4kDa
Accurate Molecular Mass: 44kDa 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.

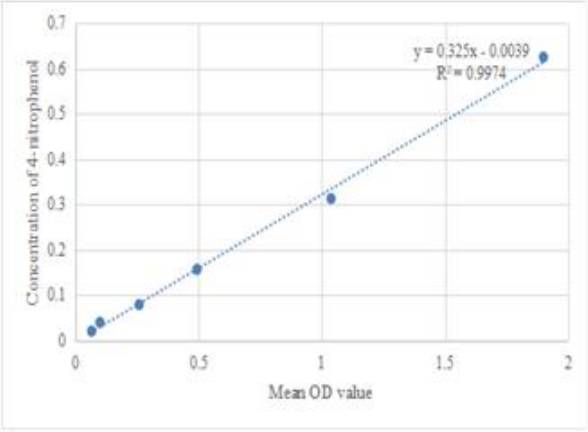
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[SEQUENCE]

PL GEEDLPSEED SPREEDPPGE EDLPGEEDLP GEEDLPEVKP
KSEEEGSLKL EDLPTVEAPG DPQEPQNNAH RDKEGDDQSH WRYGGDPPWP
RVSPACAGRF QSPVDIRPQL AAFCPALRPL ELLGFQLPPL PELRLRNNGH
SVQLTLPPGL EMALGPGREY RALQLHLHWG AAGRPGSEHT VEGHRFPAEI
HVVHLSTAFA RVDEALGRPG GLAVLAAFLE EGPEENSAYE QLLSRLEEIA
EEGSETQVPG LDISALLPSD FSRYFQYEGS LTTPPCAQGV IWTVFNQTVM
LSAKQLHTLS DTLWGPGDSR LQLNFRATQP LNGRVIEASF PAGVDSSPRA
AEPVQLNSCL AAGD

[ACTIVITY]

Carbonic Anhydrase IX (CA9) also known as membrane antigen MN and renal cell carcinoma (RCC)-associated antigen G250, is a transmembrane enzyme expressed primarily in carcinoma cells. It is one of the best markers for hypoxia and for RCC. rhCA9 corresponds to the extracellular portion of human CA9. It catalyzes the reversible reaction of $CO_2 + H_2O = HCO_3^- + H^+$, which is fundamental to many processes such as respiration, renal tubular acidification and bone resorption. The recombinant human CA9 activity 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 CA9 (diluted by Assay Buffer) to 50 μL of 2 mM Substrate 4-NPA (100 mM stock in Acetone, diluted by deionized water). Incubated at 37℃ for 5min, then read at a wavelength of 400 nm.



4-Nitrophenol (product)mM	OD400nm
0.625	1.9
0.3125	1.038
0.15625	0.494
0.078125	0.259
0.0390625	0.1
0.01953125	0.066

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 CA9 is 2554 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]

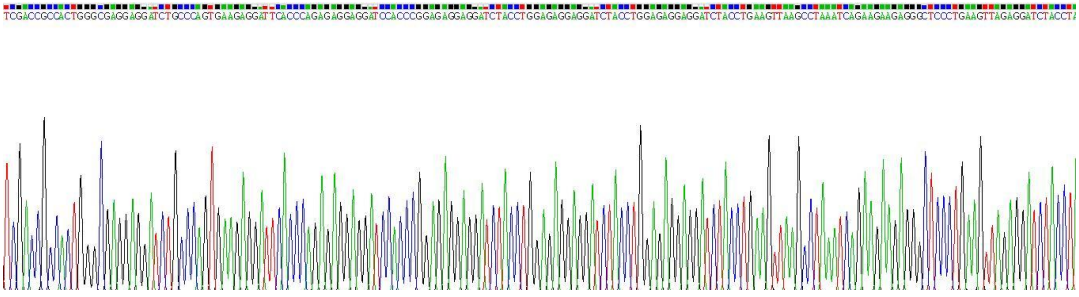


Figure 2. Gene Sequencing (extract)

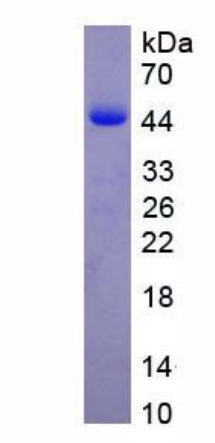


Figure 3. SDS-PAGE

Sample: Active recombinant CA9, 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.