

APD075Hu01 100µg
Active Carbonic Anhydrase VIII (CA8)
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: Met1~Gln290

Tags: N-terminal His-tag

Purity: >95%

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: 4.8

Predicted Molecular Mass: 36.7kDa

Accurate Molecular Mass: 37kDa 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.

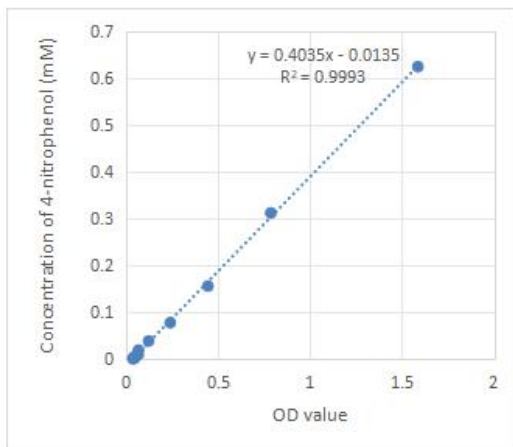
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[**SEQUENCE**]

MADLSFIEDTVAFPEKEEDEEEEEEGVEWGYEEGVEWGLVFPDANGEYQSPI
NLNSREARYDPSLLDVRLSPNYVVCRDCEVTNDGHTIQVILKSKSVLSGGPLP
QGHEFELYEVRFHWGRENQRGSEHTVNFKAFFMELHLIHWNSTLFGSIDEA
VGKPHGIAIIALFVQIGKEHVGLKAVTEILQDIQYKKGSKTIPCFNPNTLLPDPL
LRDYWVYEGSLTIPPCSEGVTWILFRYPLTISQLQIEFRRLRTHVKGAELEVC
DGILGDNFRPTQPLSDRVIRAAFQ

[**ACTIVITY**]

Carbonic Anhydrase (CA) catalyzes the reversible reaction of $\text{CO}_2 + \text{H}_2\text{O} = \text{HCO}_3^- + \text{H}^+$, which is fundamental to many processes such as respiration, renal tubular acidification and bone resorption. CA8, also called CA-related protein (CARP), is a cytosolic protein without CA activity (i.e., the reversible hydration of CO_2) due to point mutations in the zinc-binding site. CA8 is expressed exclusively in Purkinje cells of the cerebellum, where it binds inositol 1,4,5-triphosphate receptor type 1. CA8 overexpression in human colorectal cancer and non-small cell lung cancer indicates that it plays a role in the process of invasion in these types of malignancy. The activity of recombinant human CA8 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 CA8 (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°C for 5min, then read at a wavelength of 400 nm.



OD400nm	4-Nitrophenol (product) mM
1.586	0.625
0.786	0.3125
0.444	0.15625
0.239	0.078125
0.121	0.0390625
0.066	0.01953125
0.064	0.009765625
0.043	0.004882813
0.045	0.002441406
0.035	0.001220703

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 CA8 is > 900 pmol/min/ μg .

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

Δ OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

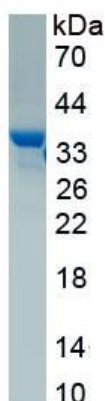


Figure 2. SDS-PAGE

Sample: Active recombinant CA8, 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.