

APA782Ra02 100µg

Active Carbonic Anhydrase II (CA2)

Organism Species: *Rattus norvegicus* (Rat)

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~Lys260

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: 6.8

Predicted Molecular Mass: 30.4kDa

Accurate Molecular Mass: 30kDa 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]

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MSHHWGYSKS NGPENWHKEF PIANGDRQSP VDI DTGTAQH DPSLQPL LIC YDKVASKSIV
NNGHSFNVEF DDSQDFAVLK EGPLSGSYRL IQFHFHWGSS DGQGSEHTVN KKKYAAELHL
VHWNTKYGDF GKAVQHPDGL AVLGIFLKIG PASQGLQKIT EALHSIKTKG KRAAFANFDP
CSLLPGNLDY WTPGSLTTP PLEECVTWIV LKEPITVSSE QMSHFRKLN F NSEGEAEELM
VDNWRPAQPL KNRKIKASFK
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[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. CA2 is a cytosolic enzyme with the highest activity among all known CAs. Mutations in the CA2 gene result in the CA II deficiency syndrome, an autosomal recessive disorder that produces osteopetrosis, renal tubular acidosis and cerebral calcification. The activity of recombinant rat CA2 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 CA2 (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.

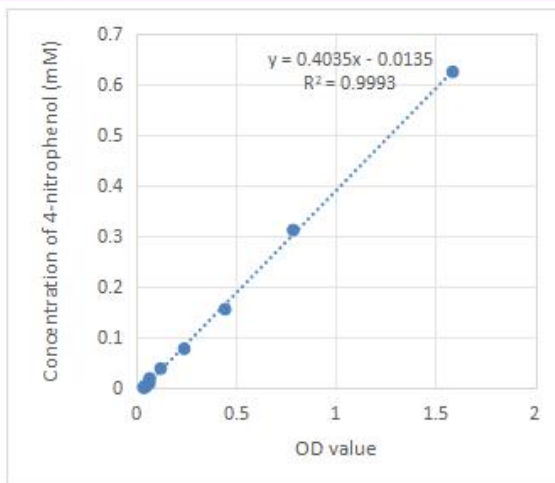


Figure 1. The standard curve of 4-Nitrophenol

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

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 rat CA2 is > 50200 pmol/min/µg.

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

△OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

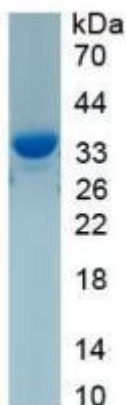


Figure 2. SDS-PAGE

Sample: Active recombinant CA2, Rat

[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.