

APD070Hu01 100µg
Active Carbonic Anhydrase III, Muscle Specific (CA3)
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: Ala2~Lys260

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: 7.4

Predicted Molecular Mass: 33.1kDa

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.

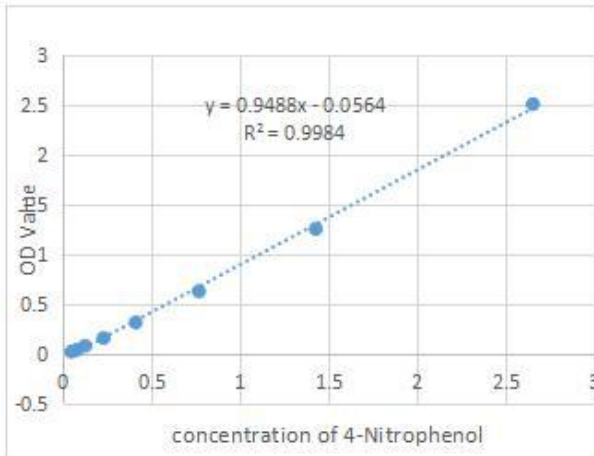
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[**SEQUENCE**]

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AKEWGYASH NGPDHWHELY PIAKGDNQSP IELHTKDIKH DPSLQPWSAS
YDPGSAKTIL NNGKTCRVVF DDTYDRSMLR GGPLSGPYRL RQFHLHWGSS
DDHGSEHTVD GVKYAAELHL VHWNPKYNTF GEALKQPDGI AVVGIFLKIG
REKGEFQILL DALDKIKTKG KEAPFTHFDP SCLFPACRDY WTYHGSFTTP
PCEECIVWLL LKEPMTVSSD QMAKLRSLFS SAENEPPVPL VGNWRPPQPV
KGRVVRASFK
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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. CA3 is a cytosolic enzyme with a very low CA activity. It is expressed at low levels in human muscle during early development but increases rapidly during the last trimester to reach 50-60% of adult levels at birth. The activity of recombinant human CA3 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 CA3 (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.



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 μ g 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 CA3 is > 17000 pmol/min/ μ g.

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

Δ OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

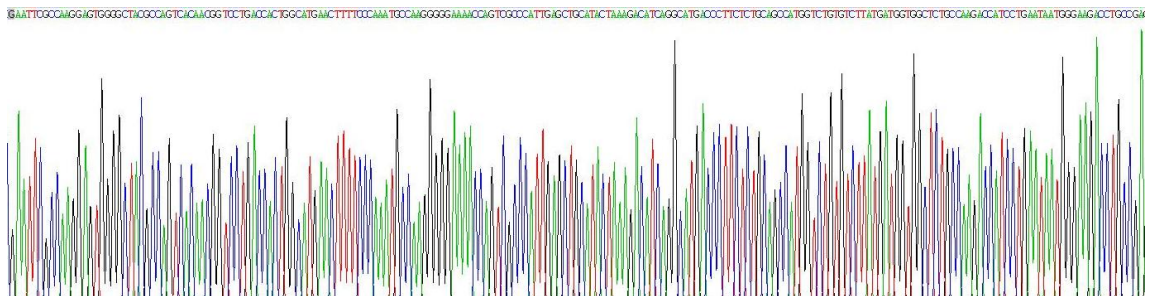


Figure 2. Gene Sequencing (extract)

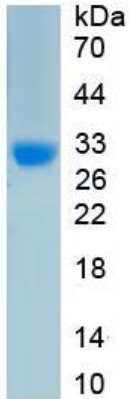


Figure 3. SDS-PAGE

Sample: Active recombinant CA3, 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.