

Background

Carbonic Anhydrase 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 (1). Topics in a CA meeting (6th International Conference on the CAs, June 20 - 25, 2003, Slovakia) ranged from the use of CAs as markers for tumor and hypoxia in the clinic, as a nutritional supplement in milk, and as a tool for CO_2 removal and mosquito control in industry. Carbonic anhydrase VA encoded by the CA5A gene is a mitochondrial protein (2, 3). In comparison with another mitochondrial CA (CA5B), CA5A has different tissue distribution and chromosomal location (4, 5). Expression and inhibitor studies of different CAs in the rat pancreatic beta cells indicate that CA5A may be involved in the regulation of insulin secretion (6). CA5A may also participate in the detoxification of ammonia produced in the gastrointestinal tract by providing bicarbonate to carbamyl phosphate synthetase I (7). The amino acid sequence of recombinant human CA5A (residues 40 to 305) is 79%, 77%, and 76% identical to that of canine, bovine, and rat/mouse.

References:

- Hewett-Emmett, D. and R.E. Tashian (1996) Mol. Phylogenet. Evol. 5:50.
- Nagao, Y. *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:7623.
- Nagao, Y. *et al.* (1995) Genomics 28:477.
- Shah, G.N. *et al.* (2000) Proc. Natl. Acad. Sci. USA 97:1677.
- Fujikawa-Adachi, K. *et al.* (1999) J. Biol. Chem. 274:21228.
- Parkkila, A.K. *et al.* (1998) J. Biol. Chem. 273:24620.
- Saarnio, J. *et al.* (1999) J. Histochem. Cytochem. 47:517.

Description

Source	<i>E. coli</i> -derived Ala40 - Ser305, with a C-terminal 10-His tag Accession # NP_001730
N-terminal Sequence Analysis	Ala40
Predicted Molecular Mass	31 kDa

Specifications

SDS-PAGE	32 kDa, reducing conditions
Activity	Measured by its esterase activity. The specific activity is > 100 pmoles/min/ μg , as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Preparation and Storage

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> 6 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after opening.

Activity Assay Protocol

Materials

- Assay Buffer: 12.5 mM Tris, 75 mM NaCl, 0.05% (v/v) Brij-35, pH 7.5
- Recombinant Human Carbonic Anhydrase VA (rhCA5A) (R&D Systems, Catalog # 3049-CA)
- Substrate: 4-Nitrophenyl acetate (4-NPA) (Sigma, Catalog # N8130), 100 mM stock in acetone
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

- Dilute rhCA5A to 20 ng/ μL in Assay Buffer.
- Dilute Substrate to 2 mM in Assay Buffer.
- In a plate, load 50 μL of 20 ng/ μL rhCA5A, and start the reaction by adding 50 μL of 2 mM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 2 mM Substrate.
- Read at a wavelength of 400 nm (bottom read) in kinetic mode for 5 minutes.
- Calculate specific activity:

$$\text{Specific Activity (pmoles/min}/\mu\text{g}) = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD}/\text{min}) \times \text{Conversion Factor}^{**} (\text{pmole}/\text{OD})}{\text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Nitrophenol (Sigma, Catalog # 241326)

Final Assay Conditions Per Well

- rhCA5A: 1 μg
- Substrate: 1 mM

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NOT FOR USE IN HUMANS.