



Recombinant Human Carbonic Anhydrase III

Catalog Number: 2185-CA

Background

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 (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. 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 (2).

References:

1. Hewett-Emmett, D. and R.E. Tashian (1996) Mol. Phylogenet. Evol. 5:50.
2. Sly, W.S. and P.Y. Hu (1995) Annu. Rev. Biochem. 64:375.

Description

Source	<i>E. coli</i> -derived Ala2 - Lys260 Accession # P07451
N-terminal Sequence Analysis	Ala2
Predicted Molecular Mass	31 kDa

Specifications

SDS-PAGE	30 kDa, reducing conditions
Activity	Measured by its esterase activity. The specific activity is > 1 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	Lyophilized from a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Preparation and Storage

Reconstitution	Reconstitute at 1 mg/mL in sterile 25 mM Tris and 150 mM NaCl, pH 7.5.
Shipping	The product is shipped at ambient temperature. 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 reconstitution.

Activity Assay Protocol

Materials

- Assay Buffer: 12.5 mM Tris, 75 mM NaCl, pH 7.5
- Recombinant human Carbonic Anhydrase III (rhCA3) (R&D Systems, Catalog # 2185-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

1. Dilute rhCA3 to 400 ng/ μL in Assay Buffer.
2. Dilute Substrate to 2.0 mM in Assay Buffer.
3. In a plate load 50 μL of 400 ng/ μL rhCA3, and include a Substrate Blank containing 50 μL Assay Buffer.
4. Start the reaction by adding 50 μL of 2.0 mM Substrate to wells containing rhCA3 and Assay Buffer.
5. Read at a wavelength of 400 nm (bottom read) in kinetic mode for 5 minutes.
6. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions Per Well

- rhCA3: 20 μg
- Substrate: 1.0 mM

10/27/2009

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NOT FOR USE IN HUMANS.