Recombinant Human Carbonic Anhydrase XII/CA12
Catalog Number: 2190-CA

DESCRIPTION

Source: Mouse myeloma cell line, NS0-derived Ala25-Gln291, with a C-terminal 10-His tag
Accession #: O43570

N-terminal Sequence Analysis: Ala25
Predicted Molecular Mass: 32 kDa

SPECIFICATIONS

SDS-PAGE: 38-41 kDa, reducing conditions
Activity: Measured by its esterase activity. The specific activity is >10 pmol/min/µg, as measured under the described conditions.
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.

Activity Assay Protocol

Materials
- Assay Buffer: 12.5 mM Tris, 75 mM NaCl, pH 7.5
- Recombinant Human Carbonic Anhydrase XII/CA12 (rhCA12) (Catalog # 2190-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 rhCA12 to 20 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. In a plate, load 50 µL of 20 ng/µL rhCA12, and start the reaction by adding 50 µL of 2 mM Substrate to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 2 mM Substrate.
4. Read absorbance at a wavelength of 400 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:
   \[ \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times (\text{OD/min}) \times \text{Conversion Factor}^{\text{**}}}{\text{amount of enzyme (µg)}} \]
   *Adjusted for Substrate Blank
   **Derived using calibration standard 4-Nitrophenol (Sigma, Catalog # 241326).

Final Assay Conditions: Per Well:
- rhCA12: 1 µg
- Substrate: 1 mM

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.
- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Carbonic Anhydrase (CA) catalyzes the reversible reaction of CO$_2$ + H$_2$O = HCO$_3^-$ + 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. CA12 is a type I membrane enzyme highly expressed in colon, kidney, prostate, intestine and activated lymphocytes and moderately expressed in pancreas, ovary, and testis (2, 3). It is over-expressed in some renal cell cancers and in glaucoma (2, 4). Two alternatively spliced forms exist, which either contains or lacks a 11 amino acid segment just before the transmembrane domain (5). The secreted, purified recombinant human CA12 contains the residues that are common for both forms.

References: