

DESCRIPTION

Source *E. coli*-derived *P. vulgaris* Chondroitinase ABC protein
Ala25-Pro1021, with an N-terminal Met and 6-His tag
Accession # P59807

N-terminal Sequence Analysis Met

Predicted Molecular Mass 113 kDa

SPECIFICATIONS

SDS-PAGE 85-100 kDa, reducing conditions

Activity Measured by its ability to hydrolyze chondroitin sulfate.
The specific activity is >15,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 μm filtered solution in Tris, NaCl and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 100 mM Tris, 50 mM NaCl, 10 mM MgCl₂, pH 7.5
 - Recombinant *P. vulgaris* Chondroitinase ABC (*rP. vulgaris* Chondroitinase ABC) (Catalog # 6877-GH)
 - Substrate: Chondroitin sulfate (Sigma, Catalog # C6737), 10 mg/mL stock in deionized water
 - 96 well UV Plate (Costar, Catalog # 3635)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute *rP. vulgaris* Chondroitinase ABC to 1 ng/μL in Assay Buffer.
 2. Dilute Substrate to 4 mg/mL in Assay Buffer.
 3. Load 50 μL of 1 ng/μL *rP. vulgaris* Chondroitinase ABC into the plate, and start the reaction by adding 50 μL of 4 mg/mL Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 4 mg/mL Substrate.
 4. Read at an absorbance of 232 nm in kinetic mode for 5 minutes.
 5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 3800 M⁻¹cm⁻¹

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD.

- Final Assay Conditions** Per Well:
- *rP. vulgaris* Chondroitinase ABC: 0.050 μg
 - Chondroitin sulfate: 200 μg

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

Chondroitinase ABC from *Proteus vulgaris* is a depolymerizing lyase that is especially active on various forms of chondroitin sulfate and dermatan sulfate (1, 2). Chondroitin sulfate contributes a major structural component of cartilage and provides much of the cartilage's resistance to compression (3). Chondroitin sulfate is also found in the nervous system and at the cell surface, where it plays regulatory roles in development (4, 5) and is involved in pathways related to microbial infection (6). Dermatan sulfate is primarily located in the skin, but is also found in the blood vessels, heart valves, tendons, and lungs, where it plays roles in blood coagulation and wound healing (7). Chondroitinase ABC is also about one hundred-fold less active on hyaluronan, a nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues (8). Chondroitinase ABC can be used to study the structures and functions of these polysaccharides. For example, in one study it was found to promote axon growth following spinal cord injury, presumably through the degradation of chondroitin sulfate in close proximity to the axons (9). Chondroitinase ABC works through β -elimination of the 1,4-hexosaminidic bond on the polysaccharides; thereby generates unsaturated disaccharides and tetrasaccharides that exhibit strong UV absorbance at 232 nm (10).

References:

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PRODUCT SPECIFIC NOTICES

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