

## Product Information

## Chondroitinase AC from *Flavobacterium heparinum*

Recombinant, expressed in *E. coli*, ≥200 units/mg protein, For Chondroitin Sulfate Analysis

**E2039**

### Product Description

CAS Registry Number: 9047-57-8

Enzyme Commission (EC) Number: 4.2.2.5

Synonyms: Chondroitin AC lyase

Chondroitinase AC from *Flavobacterium heparinum* is an eliminase that degrades chondroitin sulfates A and C, but not chondroitin sulfate B. The enzyme cleaves, via an elimination mechanism, both sulfated and non-sulfated polysaccharide chains that contain (1→4)-linkages between hexosamines and glucuronic acid residues. The reaction yields oligosaccharide products, mainly disaccharides, with unsaturated uronic acids that can be detected by UV spectroscopy at 232 nm.<sup>1,2</sup>

Chondroitinase AC has been shown to inhibit melanoma invasion and proliferation, endothelial proliferation, and angiogenesis.<sup>3</sup> Chondroitinase AC, but not chondroitinase B, has also been shown to induce apoptosis of melanoma and endothelial cells, as measured by the activity of caspase-3.<sup>3</sup>

This chondroitinase AC product has been cited in academic studies.<sup>5</sup>

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Product

#### Purity

≥90% (SDS-PAGE)

The product is essentially free of heparinase, sulfatase, heparitinase, glucuronidase, and protease activities.

#### Specific activity

≥200 units/mg protein

#### Unit definition

1 unit is defined as the amount of enzyme that will liberate 1.0 μmole per minute of unsaturated disaccharides from chondroitin sulfate A at pH 6.7 at 37 °C, as measured by the change in  $A_{232}$ . The  $\epsilon^{mM}$  for the reaction product  $\Delta$ -Di-4S (chondroitin sulfates A and B) is 5.1, and is 5.5 for  $\Delta$ -Di-6S (chondroitin sulfate C).<sup>2</sup>

The optimal pH for the assay at 37 °C is pH 6.7. The optimal chondroitin sulfate concentration in the reaction is 1 mg/mL. The activity also depends on the salt concentration and is maximal at >150 mM NaCl.

#### Relative activity of chondroitinase AC with chondroitin sulfates

- Chondroitin sulfate A: 1.0
- Chondroitin sulfate C: 0.6
- Chondroitin sulfate B: 0.03

Residual activity observed with chondroitin sulfate B may be due to small impurities in the substrate used for the assay.<sup>4</sup>

### Preparation Instructions

Reconstitute the contents of the vial with 100 μL of water to give a solution that contains ~25 mM potassium phosphate (pH 6.5), 150 mM NaCl, and a stabilizer.

## Storage/Stability

Store the product at  $-20\text{ }^{\circ}\text{C}$ . When stored properly and unopened at  $-20\text{ }^{\circ}\text{C}$ , the enzyme has a recommended retest date of 2 years.

After reconstitution, the product may be kept at  $4\text{ }^{\circ}\text{C}$  for 4 days. However, it is recommended to store the solution in working aliquots at  $-20\text{ }^{\circ}\text{C}$ .

## References

1. Saito, H. *et al.*, *J. Biol. Chem.*, **243(7)**, 1536-1542 (1968).
2. Yamagata, T. *et al.*, *J. Biol. Chem.*, **243(7)**, 1523-1535 (1968).
3. Denholm, E.M. *et al.*, *Eur. J. Pharmacol.*, **416(3)**, 213-221 (2001).
4. Aguiar, J.A.K. *et al.*, *Biotechnol. Appl. Biochem.*, **37(2)**, 115-127 (2003).
5. Ilham, Seffouh, "Analyse protéomique des endosulfatases humaines HSulfs, enzymes clés de la modulation de la sulfatation de l'héparane sulfate" ("Proteomic analysis of human endosulfatases HSulfs, key enzymes in the modulation of heparan sulfate sulfation"). Université Paris-Saclay, Ph.D. dissertation, p. 141 (2018).

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