## SIGMA-ALDRICH®

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# **Product Information**

Chondroitinase AC from *Flavobacterium heparinum* recombinant, expressed in *E. coli* 

Catalog Number **E2039** Storage Temperature –20 °C

CAS RN 9047-57-8 EC 4.2.2.5 Synonym: Chondroitin AC lyase

### **Product Description**

Chondroitinase AC from *Flavobacteriun 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  $(1\rightarrow 4)$ -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 was 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>

The enzyme is supplied as a lyophilized powder containing potassium phosphate, NaCl, and a stabilizer.

### 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  $\mu$ mole per minute of unsaturated disaccharides from chondroitin sulfate A at pH 6.7 at 37 °C, as measured by the change in A<sub>232</sub>. The  $\epsilon^{mM}$  for the reaction product  $\Delta$ -Di-4S (chondroitin sulfates A and B) is 5.1 and 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 and 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.

The relative activity of the enzyme with chondroitin sulfates A, C, and B is 1.0, 0.6, and 0.03, respectively. Residual activity observed with chondroitin sulfate B may be due to small impurities in the substrate used for the assay.<sup>4</sup>

### **Precautions and Disclaimer**

This product is 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.

### **Preparation Instructions**

Reconstitute the contents of the vial with 100  $\mu$ L of water to give a solution containing ~25 mM potassium phosphate, pH 6.5, 150 mM NaCl, and a stabilizer.

### Storage/Stability

Store the product at -20 °C. When stored properly and unopened at -20 °C, the enzyme has a recommended retest date of 2 years.

After reconstitution, the product may be kept at 4 °C for 4 days, but it is recommended to store the solution in working aliquots at -20 °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).

### RBG, DT, IG, GCY, MAM 07/16-1

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