SEIKAGAKU BIOBUSINESS CORPORATION

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CATALOG # 100330-1A
Package Size: 10 units / vial

Chondroitinase ABC (*Proteus vulgaris*)
EC 4.2.2.4 / CAS Number: 9024-13-9

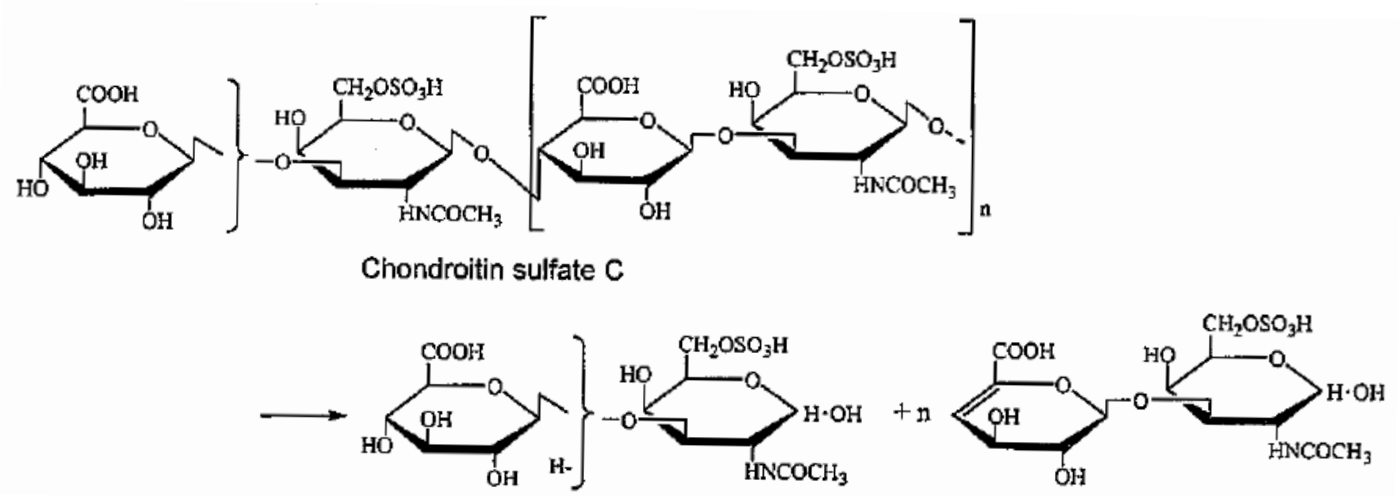
DESCRIPTION:

Chondroitin ABC lyase, Chondroitinase ABC, Chondroitin ABC eliminase.

Chondroitinase ABC purified from *Proteus vulgaris* by precipitaiton with ammonium sulfate followed by DEAE - Cellulose chromatography catalyzes the eliminative cleavage of *N* - acetylhexosaminide linkages in chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, chondroitin, and hyaluronic acid, yielding mainly disaccharides with delta 4 - hexuronate at the non - reducing ends. This enzyme does not act on keratan sulfate, heparin, and heparan sulfate. The initial rates of enzymatic degradation of chondroitin sulfate C, dermatan sulfate, chondroitin and hyaluronic acid were 1.0, 0.4, 0.2, and 0.02, respectively, relative to the rate of chondroitin sulfate A degradation.

The enzyme can be used for selective removal of the chondroitin sulfate or dermatan sulfate side chains from proteoglycans, yielding a protein - enriched core molecule[4].

REACTION (e.g. chondroitin sulfate C as substrate:



2-Acetamido-2-deoxy [-3-O-(ß-D-gluco-pyranosyluronic acid)] – 6-sulfo-D-galactise or *N*-acetylgalactosamine 6-sulfate

2-Acetamido-2-deoxy [-3-O-(ß-D-gluco-4-enepyranosyluronic acid)] – 6-sulfo-D-galactose (ΔDi-6S)

SPECIFICATIONS:

Activity:	≥10.0 units/vial	
Specific Activity:	≥50.0 units/mg protein	
Contaminants:	Chondro-4-sulfatase	≤1.0 x 10^{-2} units/vial
	Chondro-6-sulfatase	≤4.6 x 10^{-5} units/vial
	(By Morgan-Elson reaction)	
Appearance:	Lyophilised powder containing 20 mM Tris-HCl buffer, pH 7.2	
Stabilizer:	BSA free	
Preservative:	None	
Reconstitution:	Dissolve the enzyme in 200 µl of 0.1% BSA	

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Optimum pH:	8.0 (for chondroitin sulfate) 6.2 (for hyaluronic acid)
Recommended Reaction Temperature:	37°C
Molecular Weight:	120,000-145,000 (gel filtration)
Activators:	Acetate (0.05 M)
Inhibitors:	Zn^{2+} (10^{-3} M $ZnCl_2$ inhibits by 100%) Heparin (an equimolar amount of heparin inhibits by ca. 70%)

Unit Definition:
One unit is defined as the quantity of the enzyme that catalyzes the formation of 1 µmole of unsaturated disaccharide from chondroitin sulfate C (shark cartilage, see product code **400670** on the website) per minute at 37°C, pH 8.0 [1].

ASSAY FOR ENZYME ACTIVITY:

Method: The assay is based on that of Yamagata et al.[3]

Reaction mixture

Substrate and Buffer solution:	0.1 µmole chondroitin sulfate C (shark cartilage, **Cat. No. 400670** in:	
	0.4 M Tris-HCl buffer, pH 8.0	10 µl
	0.4 M sodium acetate	10 µl
	0.1% bovine serum albumin (BSA)	10 µl
	Distilled water	70 µl
Enzyme solution:	Diluted enzyme (1-5 mU) with 0.1% BSA	20 µl
Total volume:		120 µl

Procedure

Reaction:	The reaction mixture is incubated at 37°C for 10 minutes and stopped by boiling for 1 minute.
Morgan-Elson Reaction:	To enzyme reaction mixture (120 µl) add 0.1 ml of 5% $K_2B_4O_7$ (pH 9.0), and heat in a boiling water bath for 7 minutes. After cooling, add 1 ml of glacial acetic acid, mix, add 0.4 ml of the Morgan-Elson reagent, and incubate at 37°C for 20 minutes. Measure A_{585}.

Calculation:

$$\text{Enzyme Unit (units/ml)} = \frac{A_{585}}{2.38} \times \frac{0.1}{G} \times \frac{1}{10} \times \frac{1}{E}$$

where: G: Adsorption of 0.1 µmole GalNAc (A_{585})
E: Volume of enzyme solution (ml)

STORAGE:
Store at –20°C until opened. Following reconstitution, aliquot and freeze at –20°C.

REFERENCES:

1) Yamagata, T., Saito, H., Habuchi, O. And Suzuki, S. (1968) *J. Biol. Chem.*, **243**, 1523
2) Saito, H., Yamagata, T. and Suzuki, S. (1968) *J. Biol. Chem.*, **243**, 1536
3) Suzuki, S., Sato, H., Yamagata, T., Anno, K., Seno, N., Kawai, K.Y. and Furuhashi, T. (1968) *J. Biol. Chem.*, **243**, 1543
4) Oike, Y., Kimata, K., Shinomura, T., Suzuki, S., Takahashi, N. and Tanabe, K. (1982) *J. Biol. Chem*, **257**, 9751

NOTE:
For *in* vitro research use only – not for diagnostic or therapeutic use. This product is not a medical device.