SEIKAGAKU BIOBUSINESS CORPORATION

amsbio

**CATALOG # 100332-1A**
**Package Size: 2 units / vial**

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

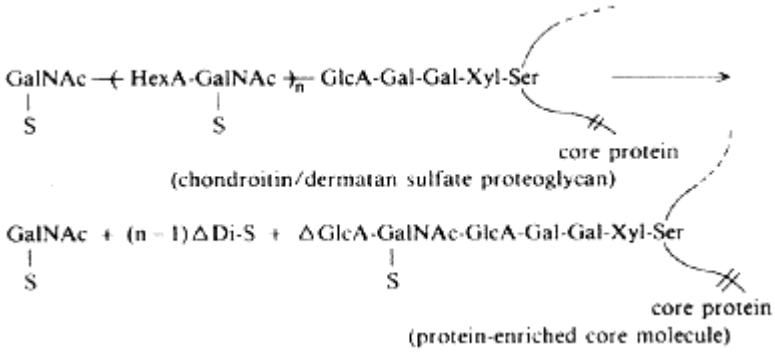
## DESCRIPTION:

Chondroitin ABC lyase, Chondroitinase ABC, Chondroitin ABC eliminase.

Chondroitinase ABC Protease Free, catalyzes the removal of chondroitin sulfate and dermatan sulfate side chains of proteoglycans, yielding protein enriched core molecules with the enzymatically modified linkage oligosaccharides[1,2]. The enzyme is highly specific for the galactosaminoglycan chains and without activity on core proteins, keratan sulfate chains, and heparin/heparan sulfate chains even in the absence of inhibitors for proteases, keratanases, and heparitinases.

Since the purity of the enzyme preparation is extremely high, and since no protein stabilizer is added, the enzyme preparation gives a main protein band with relative molecular weight of about 80,000 on SDS polyacrylamide gel electrophoresis. Because of these properties, Chondroitinase ABC Protease Free is suited for analysis and/or preparation of proteoglycan core proteins (for details of the procedures, see Ref. 1 and 2).

## REACTION:




| | | |
|---|---|---|
| Where: | GalNAc | S-*N*-acetylgalactosamine 4 (or 6)-sulfate |
| | Hex A | D-glucuronic acid or L-iduronic acid |
| | Glc A | D-glucuronic acid |
| | Gal | D-galactose |
| | Xyl | D-xylose |
| | Ser | Serine |
| | ΔGlc A | Δ4,5-glucoronic acid |
| | ΔDi-S | unsaturated sulphated disaccharides |

## SPECIFICATIONS:

| | | |
|---|---|---|
| Activity: | ≥2.0 units/vial | |
| Specific Activity: | ≥110 units/mg protein | |
| Contaminants: | Chondro-4-sulfatase | $<10^{-6}$ units/vial |
| | Chondro-6-sulfatase | $<10^{-6}$ units/vial |
| | (By Morgan-Elson reaction) | |
| | Keratanase | Not detected |
| | Heparinase | Not detected |
| | Heparatinase | Not detected |
| | (0.4 unites of Chondroitinase ABC Protease Free, 37°C, 20 hours) | |
| | Protease | Not detected |
| | (By FITC-Casein[4]) | |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| | |
|---|---|
| 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 |
| Optimum pH: | 8.0 (for chondroitin sulfate)<br>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%)<br>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.

## 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)

**Note:**
Since the enzyme action is inhibited more or less by keratan sulfate, dermatan sulfate, or heparin/heparin sulfate, amounts of the enzyme to be used should be determined by pilot tests.

## STORAGE:
Store at −70°C until opened. Following reconstitution, aliquot and freeze at −20°C.

## REFERENCES:

1) Oike, Y., Kimata, K., Shinomura, T., Nakazawa, K. and Suzuki, S. (1980) *Biochem. J.*, **191**, 193
2) Oike, Y., Kimata, K., Shinomura, T., Suzuki, S., Takahashi, N. and Tanabe, K. (1982) *J. Biol. Chem.*, **257**, 9751
3) Yamagata, T., Saito, H., Habuchi, O. And Suzuki, S. (1968) *J. Biol. Chem.*, **243**, 1523
4) Twining, S.S. (1984) *Anal. Biochem.*, **143**, 30


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


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