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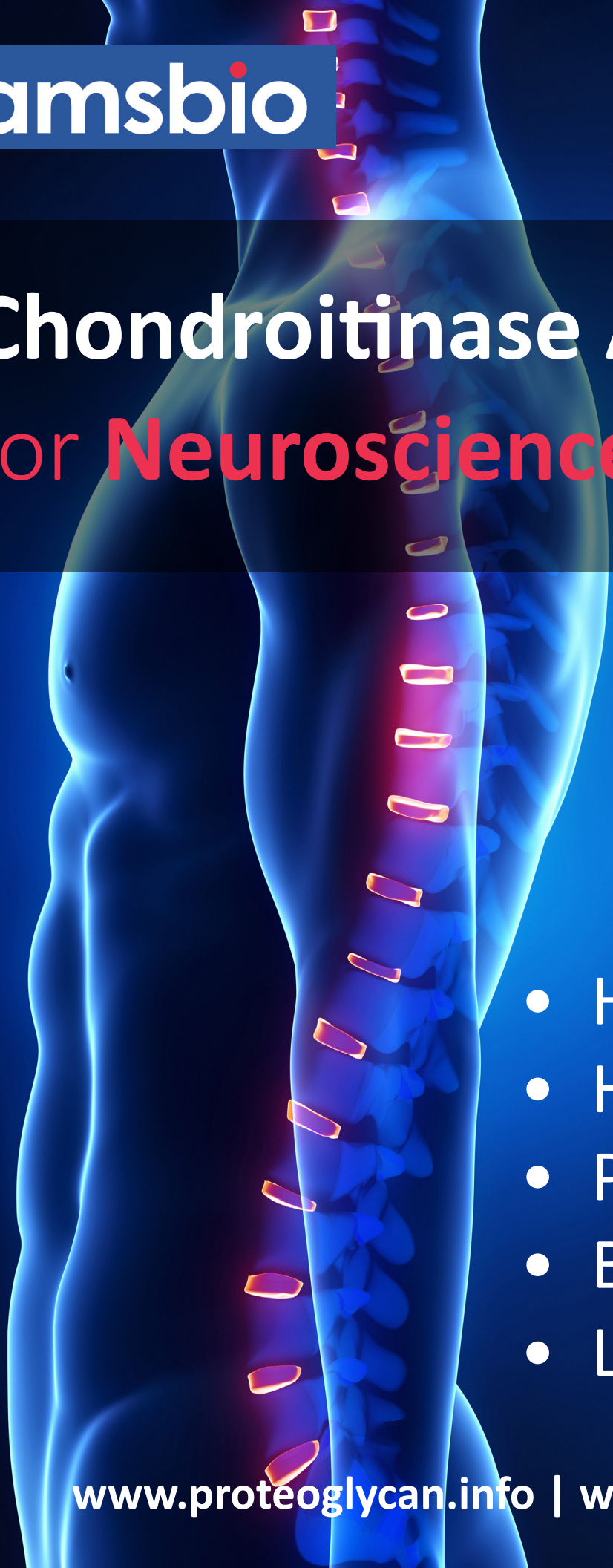
Chondroitinase ABC

for Neuroscience Research



- High Purity
- High Stability
- Protease Free
- BSA Free
- Low Endotoxin

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Chondroitinase ABC (Protease Free, BSA Free)

Chondroitinase ABC is purified from *Proteus vulgaris* by cation exchange chromatography catalyzes the degradation of chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, chondroitin and hyaluronan to mainly disaccharideswith D-4hexuronate by the eliminative cleavage of 1,4-B-hexosaminyl linkages. It does not act on keratan sulfate, heparin and heparan sulfate.

Unit Definition: One unit is defined as the amount of required to liberate 1 micro mole of unsaturated disaccharide from chondroitin sulfate C per minute at 37°C, pH8.0.

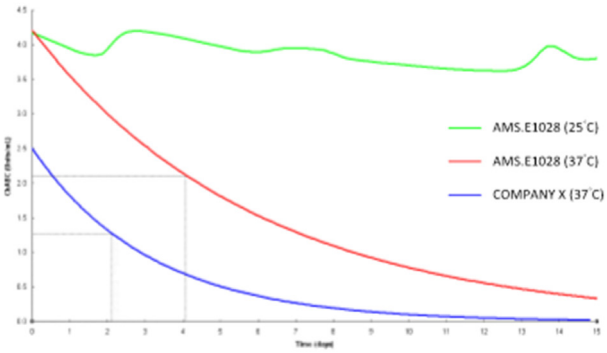
Specifications

EC Number	4.2.2.4
CAS Number	9024-13-9
Appearance:	Lyophilized powder
Specific activity:	Higher than 100units/mg protein
Endotoxin:	Specifications ≤200 EU/unit enzyme. Current batch values: <1 EU/unit enzyme
Storage:	≤-20°C
Shelf Life:	2 years
Contaminants Not Detected	Chondro-4-sulfatase, Chondro-6-sulfatase, Protease, Heparinase, Heparitinase.

High Purity

Non-reducing SDS PAGE of AMS. AMS.E1028 (left) & a competitor's ChABC (right). Samples were run in 4% stacking / 10% separating gel and stained with Coomassie Blue.

High Stability



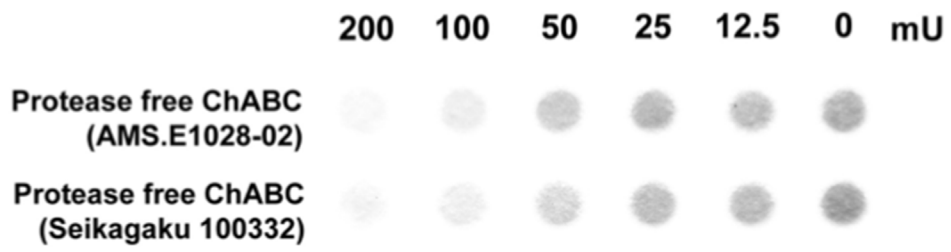
AMS.E1028 & another ChABC were reconstituted in 0.1% BSA and 5mM Ca2+-containing PBS, pH 7.2 and subsequently incubated at 37°C and 25°C. Enzyme activity measured by Morgan-Elson method.

Ordering details

Description	Cat No.	Pack Size
Chondroitinase ABC protease free (lyophilized)	AMS.E1028-02	2 Units/vial
	AMS.E1028-10	10 Units/vial

Comparison of AMS.E1028-02 with Seikagaku's Chondroitinase ABC Protease Free (100332-1A)

Assay principle: Long polysaccharide CS-A chains (200ug/ml) are set inside the gel disc which is mainly composed of 6% polyacrylamide gel. When the gel discs are incubated with the enzyme solution, the enzyme penetrates into the gel and acts on the long GAG chains. The resulting disaccharides will diffuse out of the gel due to the low gel percentage. Alcian blue is a dye which stains and binds to highly negatively charged macromolecules (i.e. the undigested polysaccharide chains remain inside the gel). In other words, the more intense colour on the gel discs, the less digestion the long GAG chains were digested with the ChABC (i.e. low activity of the enzyme).



Results: AMS.E1028-02 (top row): Intensity of the gel discs decreases with an increasing concentration of enzyme. Faint Alcian staining was observed in the discs incubated with 200 and 100mU of ChABC. Similar observation was found in the sample of #100332 (bottom row). This suggests that the ChABC from AMS.E1028-02 demonstrates similar activity to ChABC from Seikagaku #100332 (*Scientific advisor: Dr. Jessica Kwok, Fawcett Lab, Cambridge Centre for Brain Repair, Cambridge, UK*).

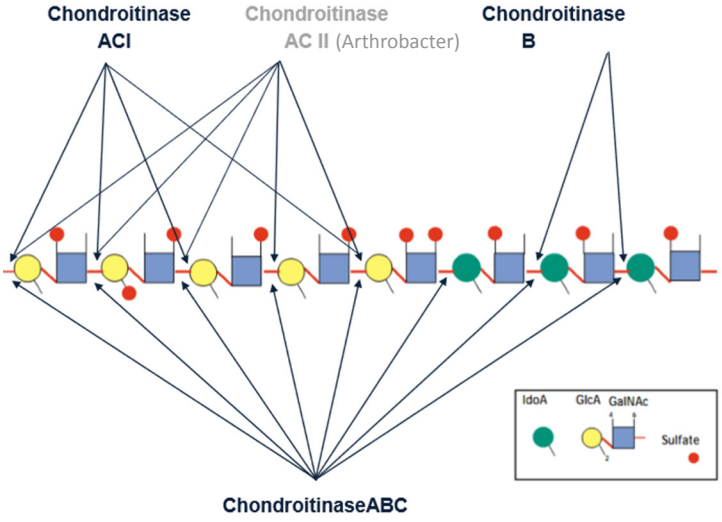
Activities of Chondroitin-Degrading Enzymes

Chondroitinase ABC Enzyme

Catalyzes the removal of Chondroitin Sulfate and Dermatan Sulfate side chains of proteoglycans. Highly specific for the galactosaminoglycan (GAG) chains without activity on core proteins, keratan sulfate chains, and heparin/ heparan sulfate chains.

Benefits

- Protease Free
- BSA Free
- low endotoxin



Description	Source	Cat No.	Pack Size
Purified			
Chondroitinase ABC protease free	*Proteus vulgaris*	AMS.E1028-02	2 U
		AMS.E1028-10	10 U
		AMS.E1028-50	50 U
Chondroitinase AC-I	*Flavobacterium heparinum*	AMS.CDACI-ENZ-S	5 IU
		AMS.CDACI-ENZ	10 IU
		AMS.CDACI-ENZ BU	20 IU
		AMS.CDACI-ENZ BU2	50 IU
		AMS.CDACI-ENZ BU3	100 IU
		AMS.CDACI-ENZ BU4	250 IU
Chondroitinase B	*Flavobacterium heparinum*	AMS.CDB-ENZ	1 IU
		AMS.CDB-ENZ BU	2 IU
		AMS.CDB-ENZ BU2	5 IU
		AMS.CDB-ENZ BU3	10 IU
		AMS.CDB-ENZ BU4	20 IU
		AMS.CDB-ENZ BU5	50 IU
Recombinant			
Chondroitinase AC, Research Grade	Recombinant *Flavobacterium heparinum*	AMS.50-013	0.5 IU

Monoclonal Chondroitin Sulfate "stub" Antibodies

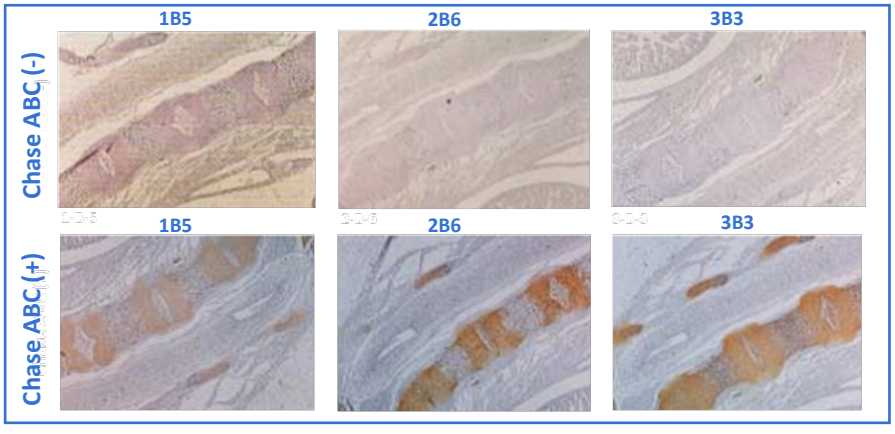
3 antibodies that specificaly recognize unsulfated (0S), 4-sulfated (4S) & 6-sulfated (6S) Chondroitin & Dermatan Sulfate, following Chondroitinase ABC digestion of various proteoglycans.

Anti Chondroitin Sulfate	Cat No.	Clone	Format	Pack Size	WB	IHC	ELISA
ΔDi-OS	270431-CS	1B5	Supernatant	1 ml	1:100	1:20	✓
ΔDi-4S	270432-CS	2B6	Supernatant	1 ml	1:100	1:20	✓
ΔDi-6S	270433-CS	3B3	Supernatant	1 ml	1:100	1:20	✓

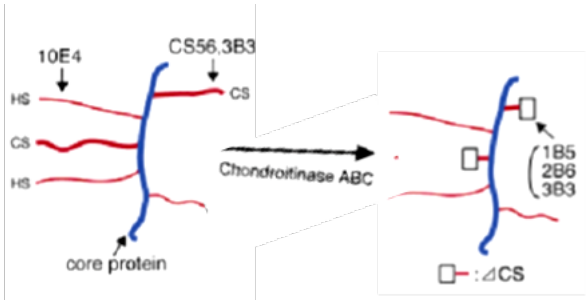
✓ = Indicates that clone has been used for this application, but no suggested dilutions available. * Optimal dilutions/ concentrations should be determined by end-user.

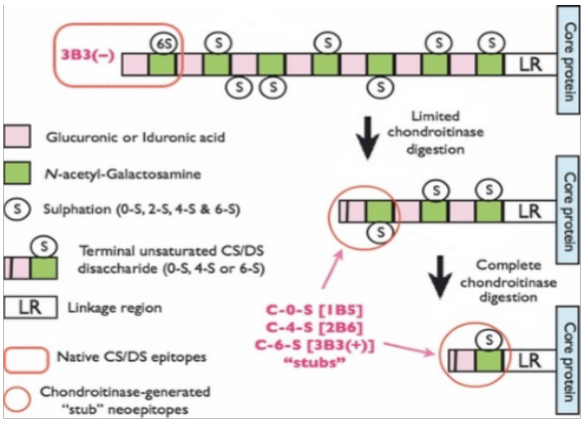
Application

Immunohistochemistry of Hamster Embryo Vertebrae, using monoclonal antibodies (1B5, 2B6, 3B3) to Chondroitin Sulfate. Showing results with & without treatment with Chondroitinase ABC (Chase ABC).



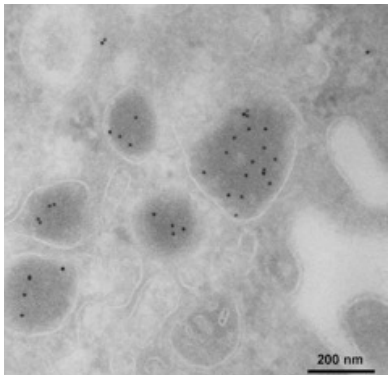
1B5: Recognises unsulfated unsaturated disaccharide neoepitopes (i.e. **C-0-S "stubs"**) generated at the non-reducing terminal of **Chondroitin Sulfate** GAG chains that have been pre-digested with either **Chondroitinase ABC** or **Chondroitinase ACI**.





Epitope mapping scheme of chondroitin sulfate-detecting antibodies. (Anti CS, clone 1B5, 2B6, 3B3).

2B6: Recognises 4-sulfated unsaturated disaccharide neoepitopes (i.e. **C-4-S "stubs"**) generated at the non-reducing terminal of Chondroitin Sulfate or Dermatan Sulfate GAG chains that have been pre-digested with Chondroitinase ABC but only Chondroitin Sulfate GAG chains pre-digested with Chondroitinase ACII or only Dermatan Sulfate GAG chains pre-digested with Chondroitinase B.

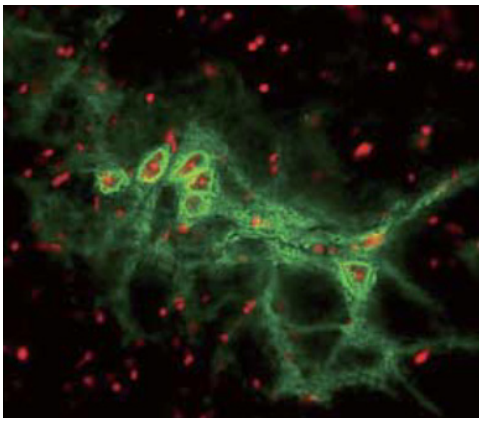


Immuno electron microscopy showing specific staining of the cytolytic granules with anti CS-4 (clone 2B6, 270432-CS). Sections were treated with 1U Chondroitinase ABC (AMS. E1028) for 2H at 37C, followed by staining with anti-CS4. Staining was detected by gold labelled Protein A. Specific staining allowed for the quantitation of granularity in primary NK cells drawn from the blood.

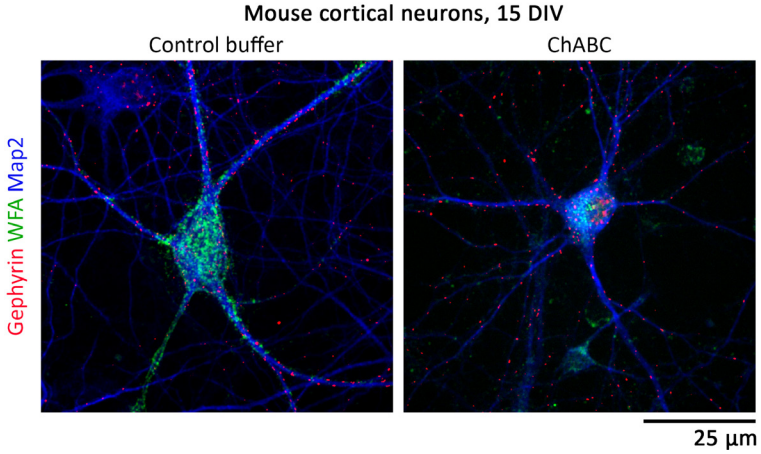
Sectioning and staining performed for Malmberg lab, Oslo University Hospital by Andreas Brech at the Norwegian Radium Hospital Institute for Cancer Research.

3B3: Recognises 6-sulfated unsaturated disaccharide neoepitopes (i.e. **C-6-S "stubs"**) generated at the non-reducing terminal of Chondroitin Sulfate GAG chains that have been pre-digested with either Chondroitinase ABC or Chondroitinase ACII. 3B3 also recognises a non-reducing end saturated disaccharide epitope in 'native' Chondroitin Sulfate GAG chains consisting of a terminal glucuronic acid adjacent to 6-sulfatedN-acetyl-galactosamine. The chondroitinase-generated neoepitope is often denoted as 3B3(+) and the 'native' terminal epitope as 3B3(-) in publications.

Perineuronal Net Removal



Perineuronal Net in Rat Cerebellum 1B5(+): Green: 1B5(+), Orange: Nuclear Staining.



Shows digestion of perineuronal net by our Chondroitinase ABC AMS.E1028 (ChABC application decreased WFA reactivity, indicating the digestion of the perineuronal net, in mouse dissociated cortical cultures).

Images courtesy of: Cell biology of the synapse laboratory, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS,
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