Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

b-Mannosidase from *Helix pomatia* Proteomics Grade

Product Number **M7819**
Storage Temperature –20 °C

CAS# 9025-43-8
EC 3.2.1.25
Synonyms: β-Mannoside mannohydrolase; β-D-Mannosidase

Product Description

β-Mannosidase is a widely used exoglycosidase enzyme in glycobiology. The enzyme cleaves single terminal D-mannosyl residues, which are β(1→4) linked to the non-reducing end of oligosaccharides (glycans) or those present on the glycan moiety of glycoproteins with relative specificity. Other mannose residues linked β(1→3) and β(1→6) are reported to be hydrolyzed at much lower rates. This property of the enzyme has been exploited in (a) the determination of the sequence and structure of the N-linked and O-linked glycans (together with other exoglycosidases) and (b) the characterization of D-galactose containing manno-oligosaccharides produced by acid or enzymatic hydrolysis of galactomannans, allowing location of D-galactosyl residues on such glycans.

Molecular weight: ~94 kDa

Isoelectric point (pI): 4.7

pH optimum: 4.0-4.5

Proteomics grade β-mannosidase has been purified to near homogeneity by several chromatographic techniques. Each lot of enzyme contains:

α-mannosidase	=0.01%
α-galactosidase	=0.01%
β-fucosidase	=0.01%
β-N-acetylglucosaminidase	=0.01%
β-galactosidase	~0.05%.

No protease activity is detected.

Unit Definition: One unit will hydrolyze 1 µmole of *p*-nitrophenyl β-D-mannopyranoside to *p*-nitrophenol (measured at 400 nm) and D-mannose per minute at pH 4.0 at 37 °C.

Components

β-Mannosidase, Proteomics Grade (Product Code M5069) Lyophilized from 10 mM sodium acetate buffer, pH 4.0, containing bovine serum albumin and NaCl	1 unit
Buffer, 5× Concentrate, for β-Mannosidase, Proteomics Grade (Product Code M6694)	2 ml

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1× Reaction Buffer is prepared by diluting an appropriate volume of the Buffer, 5× Concentrate, for β-Mannosidase, (M6694) 5-fold with deionized water and adding bovine serum albumin (BSA) to a final concentration of 0.5 mg/ml. Chill the 1× Reaction Buffer prior to use.

Prepare a β-mannosidase Stock Solution (20 units/ml) by reconstituting the β-mannosidase with 50 µl of deionized water. Re-cap the vial, mix thoroughly and centrifuge briefly before storing in aliquots at –20 °C.

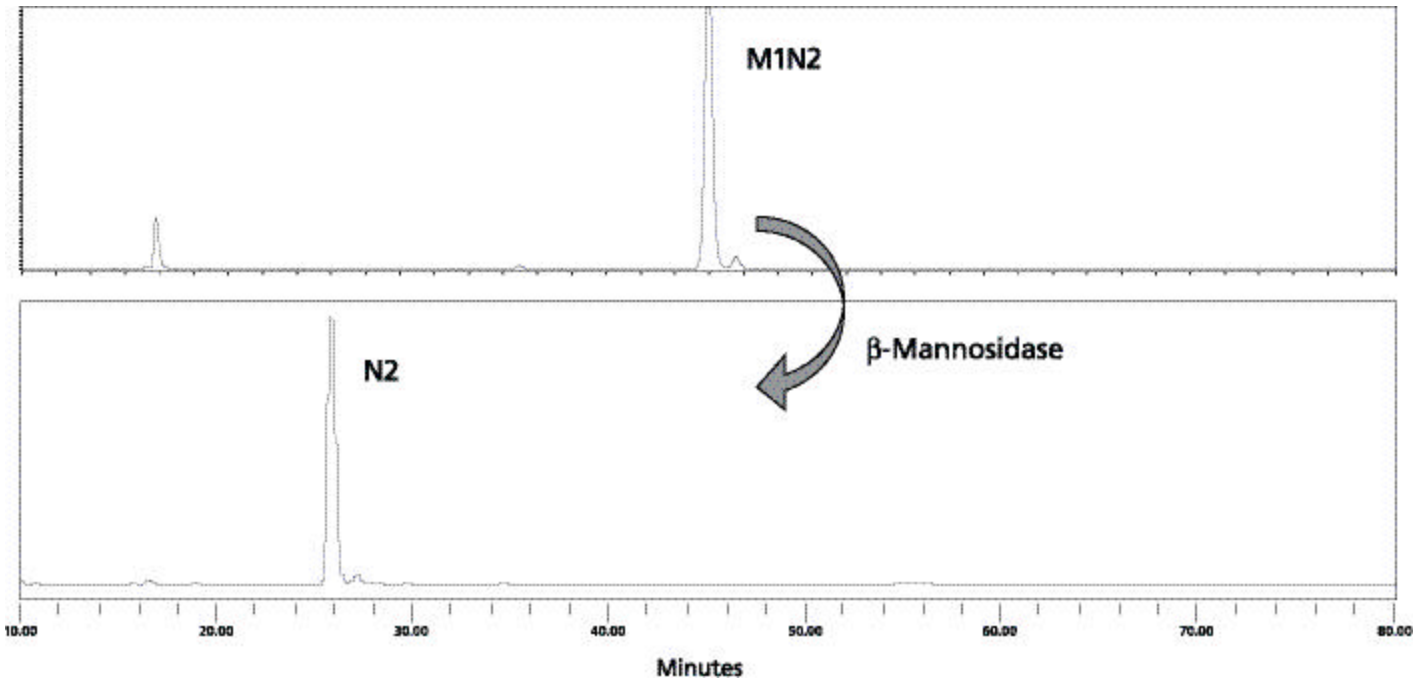
The β-mannosidase Working Solution is prepared by diluting an aliquot of the Stock Solution with an appropriate volume of cold 1× Reaction Buffer. Sigma's recommended concentration for a β-mannosidase Working Solution is minimum 4 units/ml. Working dilutions should be made in 1× Reaction Buffer.

Storage/Stability
Store the lyophilized enzyme at –20 °C. The Buffer, 5× concentrate, may be stored at 2–8 °C for convenience. The reconstituted enzyme should be aliquoted and stored at –20 °C. Avoid repeated freeze-thaw cycles.

Procedure
One of the applications of this enzyme is to remove non-reducing terminal β(1→4) linked mannose residues from the core structure of N-linked glycans. A final concentration of 2–4 units/ml of β-mannosidase is recommended for reactions containing 10–20 µM of glycan.

Results
Fluorescently labeled M1N2 glycan was incubated with β-mannosidase for 20 hours at 37 °C and then analyzed by normal phase HPLC. The data presented in Figure 1 show the shift in the retention time of the peak confirming that M1N2 glycan has been cleaved to N2 glycan with the loss of the terminal β(1→4) linked mannose residue.

Figure 1.
HPLC chromatograms demonstrating cleavage of the terminal mannose residue of M1N2 glycan by Proteomics Grade β-Mannosidase. Approximately 100 pmol of M1N2 was digested with β-mannosidase at a concentration of 3 units/ml for 20 hours at 37 °C.



References

1. McCleary, B.V., β-D-Mannosidase from *Helix pomatia.* Carbohydr. Res., **111**, 297-310 (1983).
2. McCleary, B.V., Meth. Enzymol., **160**, 614-619 (1988).
3. Charrier, M., and Rouland, C., Mannan-degrading enzymes purified from the crop of the brown garden snail *Helix aspersa* Muller (Gastropoda Pulmonata). J. Exp. Zool., **290**, 125-135 (2001).

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