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ProductInformation

α-Mannosidase
from *Canavalia ensiformis* (Jack bean)
Proteomics Grade

Product Number **M7944**
Storage Temperature 2–8 °C

CAS# 9025-42-7
EC 3.2.1.24
Synonym: α-D-Mannoside mannohydrolase

Product Description

α-Mannosidase is a widely used exoglycosidase enzyme in glycobiology and is thought to be involved in the turnover of N-linked glycans. The enzyme cleaves terminal α-D-mannosyl residues which are α(1→2,3,6) linked to the non-reducing end of oligosaccharides or those present on the glycan moiety of glycoproteins. α(1→3) Linked mannose residues are reported to be hydrolyzed at a lower rate than α(1→2) and α(1→6) linked residues. This property of the enzyme has been exploited in the determination of linkage of mannose in glycoproteins. The enzyme has been shown to cleave mannose from the glycans of ovomucoid, orosomucoid, ovalbumin, and other glycoproteins.

Molecular weight: ~220 kDa

Isoelectric point (pI): 6.2

pH optimum: 4.5

Inhibitors: Ag^{+} and Hg^{+2}

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

β-mannosidase	≤0.01%
α-galactosidase	≤0.01%
β-fucosidase	≤0.01%
β-N-acetylglucosaminidase	≤0.05%

No protease activity is detected.

Unit Definition: One unit will hydrolyze 1 µmole of *p*-nitrophenyl α-D-mannopyranoside to *p*-nitrophenol (measured at 405 nm) and D-mannose per minute at pH 4.5 at 37 °C.

Components

α-Mannosidase, Proteomics Grade (Product Code M6944) Solution in 20 mM Tris-HCl buffer containing 25 mM NaCl, pH 7.5	10 units
Buffer for α-Mannosidase, Proteomics Grade, 5× Concentrate (Product Code B1811)	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

Dilute the Buffer for α-Mannosidase, 5× Concentrate (M6694) 5-fold with deionized water to prepare a 1× reaction buffer. Dilute the α-mannosidase with 1× reaction buffer to obtain appropriate concentration (units/ml).

Storage/Stability

Store the enzyme and 5× concentrate buffer at 2–8 °C.

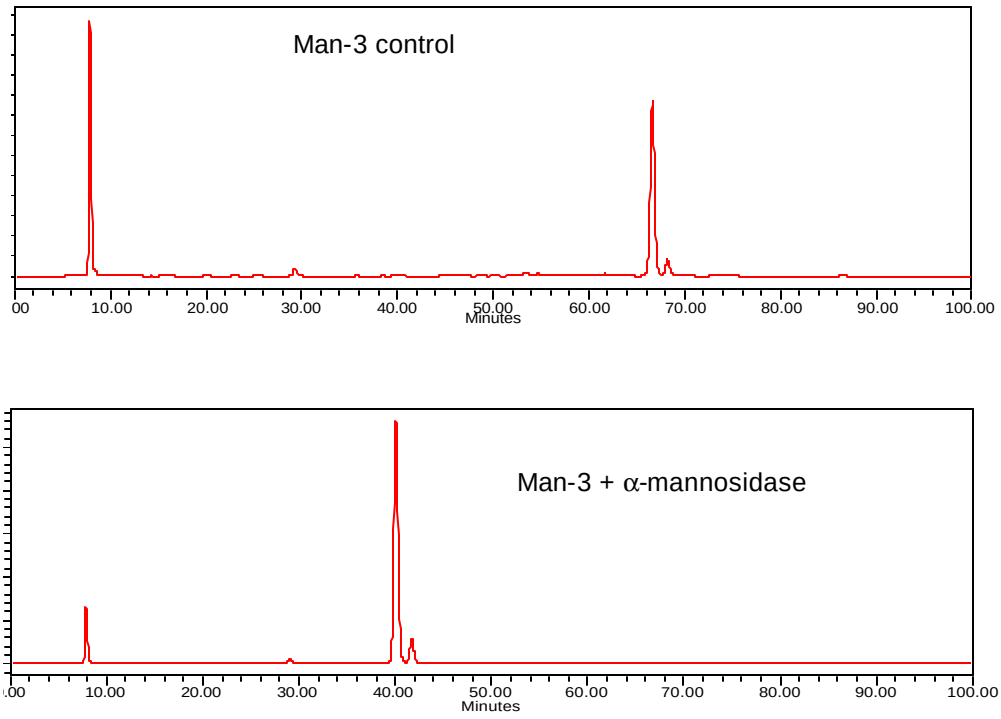
Procedure

One of the applications of this enzyme is to remove non-reducing terminal mannose residues from N-linked glycans. A concentration of 10-50 units/ml of α-mannosidase is recommended for reactions containing 10-20 µM of glycan.

Results

Fluorescently labeled Man-3 glycan was incubated with α-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 from 66 minutes to 40 minutes, confirming that Man-3 glycan has been cleaved to M1N2 glycan with the loss of two terminal [α(1→3) and α(1→6)] linked mannose residues.

Figure 1.
HPLC chromatograms demonstrating cleavage of the terminal mannose residues of Man-3 glycan by Proteomics Grade α-mannosidase. Approximately 200 pmol of Man-3 glycan was digested with α-mannosidase at a concentration of 10 units/ml for 20 hours at 37 °C.



References

1. Li, Y.T., Studies on the glycosidases in jack bean meal. I. Isolation and properties of α-mannosidase. J. Biol. Chem., **242**, 5474-5480 (1967).
2. Li, Y.T., and Li, S.C., Studies on the glycosidases in jack bean meal. II. Separation of various glycosidases by isoelectric focusing. J. Biol. Chem., **243**, 3994-3996 (1968).
3. Li, Y.T., Presence of α-D-mannosidic linkage in glycoproteins. J. Biol. Chem., **241**, 1010-1012 (1966).
4. Kimura, Y., *et.al*., The N-glycans of jack bean α-mannosidase. Structure, topology and function. Eur. J. Biochem., **264**, 168-175 (1999).

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