



β-MANNOSIDASE from *Cellulomonas fimi* (Lot 30901)

E-BMOSCF

09/03

PROPERTIES

(1) ELECTROPHORETIC PURITY

- single band on SDS-gel electrophoresis (MW ~ 103,000)
- broad diffuse band on Isoelectric focusing (pI ~ 4.8)

(2) SPECIFIC ACTIVITY AND LEVEL OF OTHER ACTIVITIES

One unit of enzyme activity is defined as the amount of enzyme required to release one μmole of *p*-nitrophenol from *p*-nitrophenyl β-mannoside (1 mM in assay) in one minute at 35°C and pH 6.5. Other glycosidase activities were measured using the appropriate *p*-nitrophenyl glycoside (at 5 mM concentration in assay), and *endo*-glycanase activities were determined with the appropriate substrate (10mg/ml) and the Nelson/Somogyi reducing sugar procedure.

ENZYME ACTIVITY	SUBSTRATE	ACTIVITY (U/mg protein)
β-Mannosidase	<i>p</i> -NP-β-D-Mannopyranoside	17.4
-Galactosidase	<i>p</i> -NP- -D-Galactopyranoside	0.0001
-Glucosidase	<i>p</i> -NP- -D-Glucopyranoside	0.0008
β-Glucosidase	<i>p</i> -NP-β-D-Glucopyranoside	0.001

One Unit of activity is the amount of enzyme required to release one μmole of *p*-nitrophenol from the appropriate nitrophenyl glycoside (0.8 mM in the assay) in 100 mM sodium maleate buffer (pH 6.5) at 35°C.

(3) RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES

SUBSTRATE	RELATIVE HYDROLYSIS RATE
Mannobiose	41
Mannotriose	98
Mannotetraose	100
Mannopentaose	95
Mannohexaose	100
Mannobitol	1.1
Mannotriitol	97
Mannotetraitol	98
6 ¹ - -D-Galactosyl-mannobiose	1.0
6 ¹ - -D-Galactosyl-mannotriose	19.5
1,4-β-D-Mannosyl-D-glucose	34
<i>p</i> -Nitrophenyl β-D-Mannopyranoside	664

Activities on all oligosaccharides were determined at a final substrate concentration of 10 mM in the reaction mixture in 100 mM sodium maleate buffer (pH 6.5) at 35°C. Action on *p*-NP-β-mannopyranoside was determined at a final substrate concentration of 0.8 mM (due to substrate inhibition; see Figure 1).

(4) TEMPERATURE AND pH STABILITY/OPTIMUM

pH Optimum:	6.5	Temperature Optimum:	35°C
pH Stability:	4.0-9.0	Temperature Stability:	Unstable above 35°C Stable at 35°C for > 6 hr.

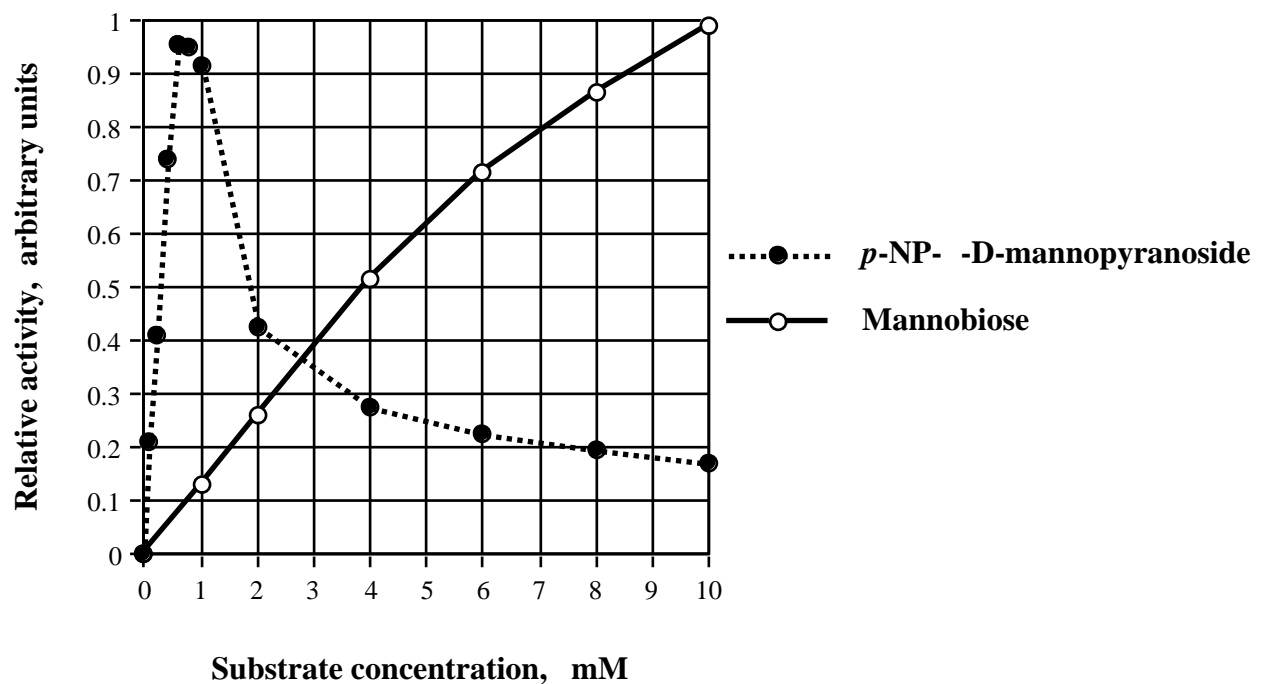


Figure 1. Effect of substrate concentration on the determined activity of α -mannosidase.

At substrate concentrations above 0.8 mM, α -mannosidase is inhibited by *p*-nitrophenyl α -D-mannopyranoside. In contrast, there is no inhibition by α -D-mannobiose at concentrations up to 10 mM in the assay mixture.

Figure 2. SDS-PAGE analysis of α -mannosidase (*Cellulomonas fimi*)

Electrophoresis was performed using a 10% acrylamide gel. Lane 1, low molecular weight markers (Sigma cat.no. M-3918); lane 2, 5 μ g α -mannosidase; lane 3, high molecular weight markers (Sigma cat. no. M-3788).

