β-MANNOSIDASE from *Cellulomonas fimi* (Lot 150501b)

Recombinant

E-BMOSCF **01/21**

(EC 3.2.1.25) *beta*-D-mannoside mannohydrolase

CAZy Family: GH2

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:

7 U/mg protein (on pNP-β-D-mannopyranoside) at pH 6.5 and 35°C.

***One Unit** of β-D-mannosidase activity is defined as the amount of enzyme required to release one µmole of *p*-nitrophenol per minute from *p*NP-β-D-mannopyranoside (0.8 mM) in sodium maleate buffer (100 mM) pH 6.5 and 35°C, monitored at 400 nm.

** Extinction coefficient (ε) of p-nitrophenol = 4330 M^{-1} x cm^{-1}*

3. OTHER ACTIVITIES (as a percentage of β-D-mannosidase activity):

Enzyme Activity	Substrate	%
β-Mannosidase	*p*-NP-β-D-Mannopyranoside	100
α-Galactosidase	*p*-NP-α-D-Galactopyranoside	< 0.001
α-Glucosidase	*p*-NP-α-D-Glucopyranoside	< 0.001
β-Glucosidase	*p*-NP-β-D-Glucopyranoside	< 0.001

Action on *p*-NP-substrates was determined at a final substrate concentration of 0.8 mM in sodium maleate buffer (100 mM), pH 6.5 at 35°C.

4. RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES:

Substrate	%
Mannobiose	41
Mannotriose	98
Mannotetraose	100
Mannopentaose	95
Mannohexaose	100
Mannobiitol	1.1
Mannotriitol	97
Mannotetraitol	98
6^1-α-D-Galactosyl-mannobiose	1.0
6^1-α-D-Galactosyl-mannotriose	19.5
1,4-β-D-Mannosyl-D-glucose	34
p-Nitrophenyl β-D-Mannopyranoside	664

Action on oligosaccharide substrates was determined at a final substrate concentration of 10 mM in sodium maleate buffer (100 mM), pH 6.5 at 35°C. Action on *p*-NP-β-D-mannopyranoside was determined at a final substrate concentration of 0.8 mM (due to substrate inhibition; see Figure 1).

5. PHYSICOCHEMICAL PROPERTIES:

pH Optima:	6.5
Temperature Optima:	35°C
pH Stability:	4.0 - 9.0
Temperature Stability:	up to 35°C (Stable at 35°C for > 6 h)

6. STORAGE CONDITIONS:

The enzyme is supplied as an ammonium sulphate suspension in 0.02% (w/v) sodium azide and should be stored at 4°C. For assay, this enzyme should be diluted in sodium maleate buffer (100 mM), pH 6.5 containing 1 mg/mL BSA. **Swirl to mix the enzyme immediately prior to use.**

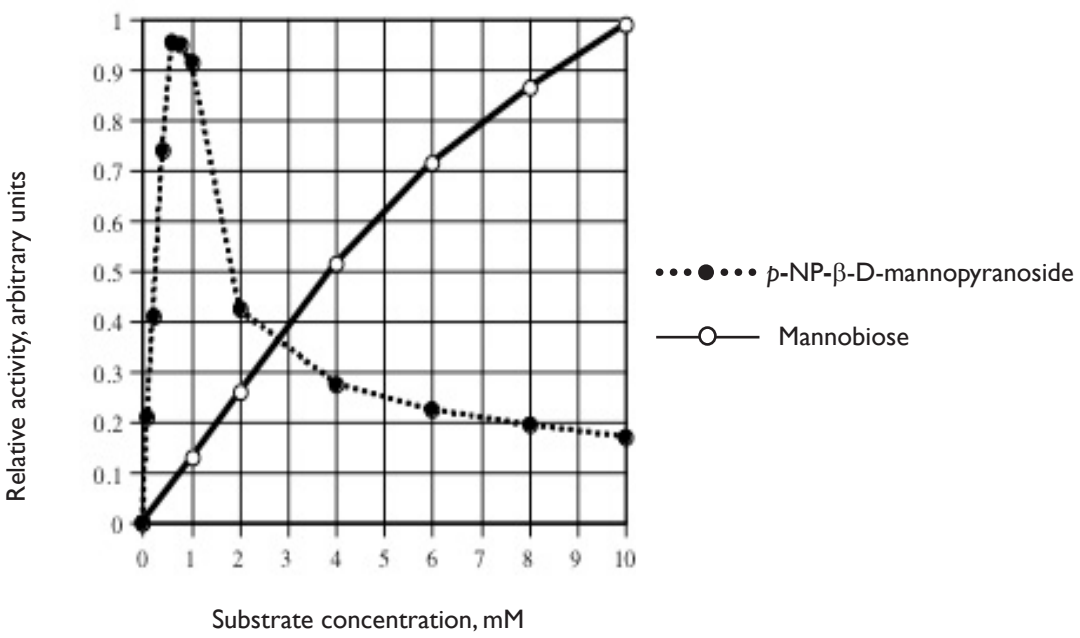

Figure 1. **Effect of substrate concentration on the determined activity of β-mannosidase.**
At substrate concentrations above 0.8 mM, β-mannosidase is inhibited by *p*-nitrophenyl β-D-mannopyranoside. In contrast, there is no inhibition by β-D-mannobiose at concentrations up to 10 mM in the assay mixture.

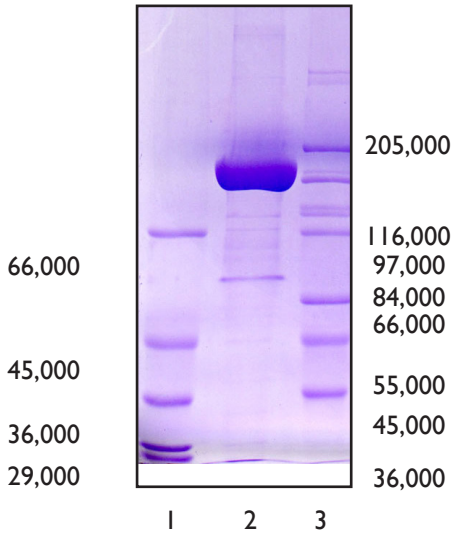

Figure 2. **SDS-PAGE analysis of β-mannosidase (*Cellulomonas fimi*)**
Electrophoresis was performed using a 10% acrylamide gel.
Lane 1: low molecular weight markers (Sigma cat. no. M-3918); **Lane 2:** 5µg β-mannosidase; **Lane 3**: high molecular weight markers (Sigma cat. no. M-3788).