



## β-MANNOSIDASE from *Cellulomonas fimi* (Lot 150501a)

### Recombinant

#### E-BMOSCF

10/17

(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 ( $\epsilon$ ) of *p*-nitrophenol =  $4330 \text{ M}^{-1} \times \text{cm}^{-1}$

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

Enzyme Activity	Substrate	%
β-Mannosidase	<i>p</i> -NP-β-D-Mannopyranoside	100
α-Galactosidase	<i>p</i> -NP-α-D-Galactopyranoside	< 0.001
α-Glucosidase	<i>p</i> -NP-α-D-Glucopyranoside	< 0.001
β-Glucosidase	<i>p</i> -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
Mannobiotol	1.1
Mannotriitol	97
Mannotetraitol	98
6 <sup>I</sup> -α-D-Galactosyl-mannobiose	1.0
6 <sup>I</sup> -α-D-Galactosyl-mannotriose	19.5
1,4-β-D-Mannosyl-D-glucose	34
<i>p</i> -Nitrophenyl β-D-Mannopyranoside	664

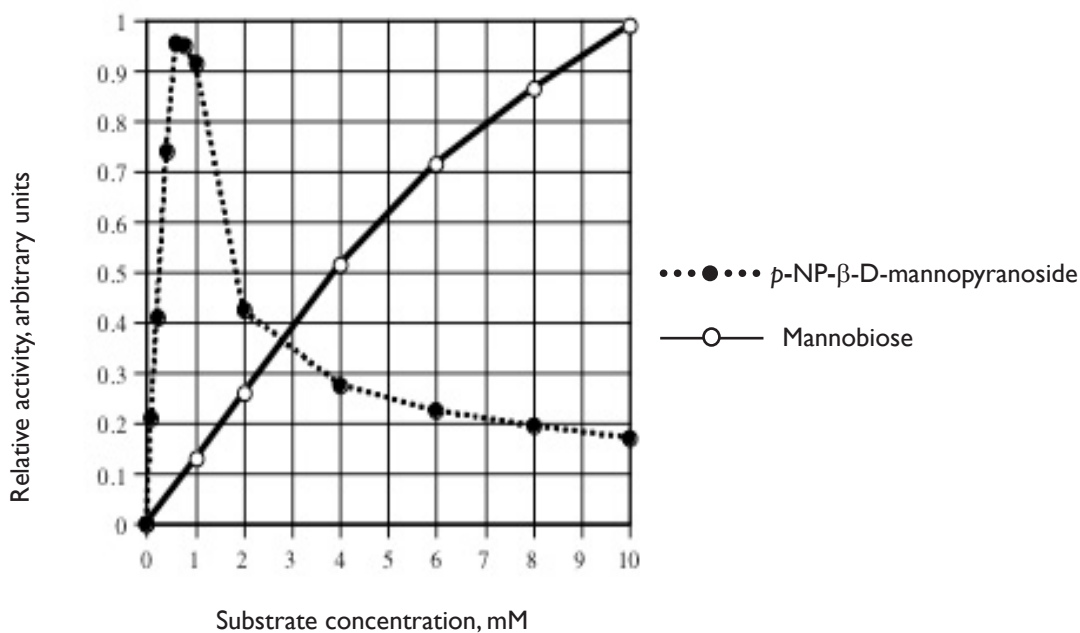
Action on oligosaccharide substrates was determined at a final substrate concentration of 10 mM in sodium maleate buffer (100 mM), pH 6.5 at 35°C. Action on *p*-NP-β-D-mannopyranoside was determined at a final substrate concentration of 0.8 mM (due to substrate inhibition; see Figure 1).

## 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)

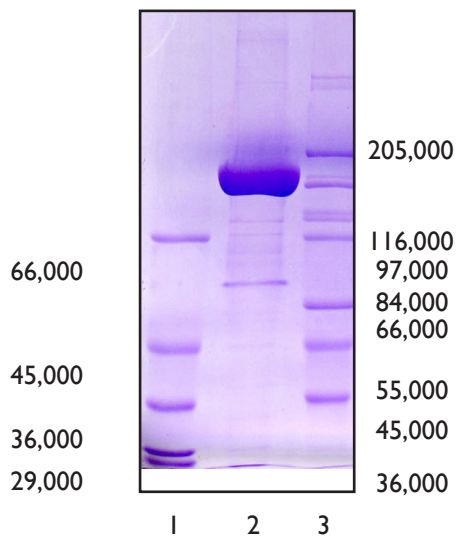
## 6. STORAGE CONDITIONS:

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:** 5 μg β-mannosidase; **Lane 3:** high molecular weight markers (Sigma cat. no. M-3788).