Endoglycosidase F1

Endoglycosidase F1 [Endo-β-N-acetylglucosaminidase F1, EC 3.2.1.96] cleaves asparagine-linked or free oligomannose and hybrid, but not complex oligosaccharides (see Figure 1). Core fucosylation reduces the activity by 50 fold. Endoglycosidase F1 will hydrolyze sulfate containing high-mannose chains. It cleaves between the two N-acetylglucosamine residues in the diacetylmaltotriose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact.

Endoglycosidase F1 is less sensitive to protein conformation than PNGase F and is therefore more suitable for deglycosylation of native proteins. However for optimal results, denaturation of the glycoprotein is recommended.

Endoglycosidase F1 is isolated from a strain of E. coli expressing a cloned gene from Elizabethkingia (Flavobacterium) meningosepticum.

Specifications
Activity
\[ \geq 16 \text{ U/mg}, \geq 17 \text{ U/mL} \]

Storage
Store at 4°C. Do not freeze.

Formulation
The enzyme is provided as a sterile solution in 20 mM Tris HCl, pH 7.5.

Stability
Stable at least 12 months when stored properly. Several days exposure to ambient temperature will not reduce activity.

Product Description
Molecular Weight
32,000 Daltons

Purity
Endoglycosidase F1 is tested for contaminating protease as follows; 10 µg of denatured BSA is incubated for 24 hours at 37°C with 2 µL of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

Specificity
Asparagine-linked hybrid or free hybrid or high mannose oligosaccharides.

Assay
One unit of Endoglycosidase F1 activity is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 µmole of denatured Ribonuclease B in 1 minute at 37°C, pH 5.5. Cleavage is monitored by SDS-PAGE (cleaved Ribonuclease B migrates faster).

Reagents
- 5X Reaction buffer 5.5 - 250 mM sodium phosphate pH 5.5
- Denaturation Solution: (2% SDS, 1 M β-mercaptoethanol [β-ME])
- Triton X-100 solution*, 15%

Suggestions for Use
Procedure for Deglycosylation
1. Add up to 200 µg of glycoprotein to Eppendorf tube.
2. Add deionized water to a total of 33 µL.
3. Add 10 µL 5X Reaction Buffer, 5.5
4. Add 2.5 µL of Denaturation Solution. Heat at 90°C for 10 minutes
5. Cool to room temperature and add 2.5 µL Triton X-100 solution
6. Add 2 µL of Endoglycosidase F1. Incubate 1 hour or more at 37°C
7. Monitor cleavage by SDS-PAGE
For digestion of native proteins, add water to a total volume of 38 µL and omit steps 4 and 5. Increase incubation time appropriately.

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References


Figure 1 - Cleavage of oligosaccharides by Endoglycosidase F1 and PNGase F

**Figure 1**

![Cleavage of oligosaccharides by Endoglycosidase F1 and PNGase F](image)

**Order Information**

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(Elizabethkingia meningosepticum recombinant)