



## Endoglycosidase F3

Endoglycosidase F3 [Endo- $\beta$ -N-acetylglucosaminidase F3, EC 3.2.1.96] cleaves asparagine-linked or free biantennary and triantennary complex, oligosaccharides depending on the state of core fucosylation and peptide linkage (see Figure 1). It cleaves between the two N-acetylglucosamine residues in the diacetylchitobiose 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. There is no activity on oligomannose and hybrid molecules.

Endoglycosidase F3 will also cleave trimannosyl-chitobiose core structures (see Figure 1). This activity was previously attributed only to Endoglycosidase D from *Streptococcus (formerly Diplococcus) pneumoniae*.

Endoglycosidase F3 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 F3 is isolated from a strain of *E. coli* expressing a cloned gene from *Elizabethkingia (Flavobacterium) meningosepticum*. The recombinant protein is not glycosylated. This alteration may result in properties that differ from the natively-derived protein.

### Specifications

#### Activity

$\geq 25$  U/mg,  $\geq 5$  U/mL

#### Storage

Store at 4°C. Do not freeze.

#### Formulation

The enzyme is provided as a sterile-filtered 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.

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### Product Description

#### Molecular Weight

30,000 Daltons

#### Purity

Each lot of Endoglycosidase F3 is tested for contaminating protease as follows: 10  $\mu$ g of denatured BSA is incubated for 24 hours at 37°C with 2  $\mu$ L 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 bi- and triantennary oligosaccharides, depending on core fucosylation and peptide linkage.

#### Assay

One unit of Endoglycosidase F3 activity is defined as the amount of enzyme required to catalyze the release of 1  $\mu$ mole of N-linked oligosaccharides from porcine fibrinogen glycopeptides<sup>3</sup> in 1 minute at 37°C, pH 4.5.

#### Reagents

- 5X Reaction buffer 4.5 - 250 mM sodium acetate pH 4.5
- Denaturation Solution - w/v sodium lauryl sulfate, 1 M  $\beta$ -mercaptoethanol
- Triton X-100 solution\*, 15% v/v Triton X-100

### Suggestions for Use

#### Procedure for Deglycosylation

1. Add up to 200  $\mu$ g of glycoprotein to Eppendorf tube.
2. Add deionized water to a total of 33  $\mu$ L.
3. Add 10  $\mu$ L 5X Reaction Buffer, 4.5
4. Add 2.5  $\mu$ L of Denaturation Solution. Heat at 90°C for 10 minutes
5. Cool to room temperature and add 2.5  $\mu$ L Triton X-100 solution
6. Add 2  $\mu$ L of Endoglycosidase F3. Incubate 1 hour or more at 37°C

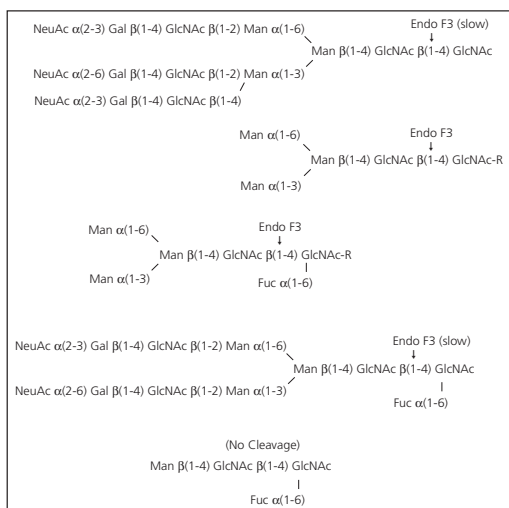
## 7. Monitor cleavage by SDS-PAGE

For digestion of native proteins, add water to a total volume of 38  $\mu$ L and omit steps 4 and 5. Increase incubation time appropriately.

## References

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**Figure 1 - Cleavage of oligosaccharides by Endoglycosidase F3**



Man = Mannose; Gal = Galactose; GlcNAc = N-acetylglucosamine; Fuc = Fucose; NeuAc = N-acetylneuraminic acid; R = Peptide linkage required

## Order Information

| Catalog No. | Product Description   | Package Size | Temp. °C |
|-------------|---|--------------|----------|
| 100463-1    | Endoglycosidase F3<br>( <i>Elizabethkingia meningosepticum</i> recombinant) | 60 $\mu$ L   | +4       |



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