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Endoglycosidase F2

Endoglycosidase F2 [Endo-β-N-acetylglucosaminidase F2 EC 3.2.1.96] cleaves asparagine-linked or free oligomannose and biantennary complex, oligosaccharides (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.

Endoglycosidase F2 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 F2 is isolated from a strain of E. coli expressing a cloned gene from *Elizabethkingia miricola*.

Product Code: GE 48

Specifications

Activity: ≥20 U/mg, ≥ 5 U/mL

Storage: Store at 4°C. Do not freeze.

Formulation: The enzyme is provided as a sterile solution in 10 mM Sodium Acetate and 25 mM Sodium Chloride pH 4.5.

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

Product Description

Molecular weight: 32,000 daltons

Purity: Endoglycosidase F2 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 high mannose oligosaccharides.

Assay

One unit of Endoglycosidase F2 activity is defined as the amount of enzyme required to catalyze the release of 1 μmole of N-linked oligosaccharides from porcine fibrinogen glycopeptides 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 β-mercaptoethanol
- Triton X-100 solution*, 15% v/v TritonX-100

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, 4.5.

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 F2. 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.

References

1. Maley P., R. B. Trimble, A. L. Tarentino and T. H. Plummer Jr. Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases. **Anal Biochem 180:**195-204 (1989).

2. Plummer, T. H. Jr, A. W. Phelan and A. L. Tarentino. Porcine fibrinogen glycopeptides: substrates for detecting endo-beta-N-acetylglucosaminidases F2 and F3**. Anal Biochem 235:**98-101 (1996).

3. Reddy A., B. G. Grimwood, T. H. Plummer Jr and A. L. Tarentino. High-level expression of the Endo-beta-N-acetylglucosaminidase F2 gene in E.coli: one step purification to homogeneity. **Glycobiology 8:**633-636 (1998).

4. Tarentino, A. L., C. M. Gomez and T. H. Plummer Jr. Deglycosylation of Asparagine-Linked Glycans by Peptide:N-Glycosidase F. **Biochemistry 24:**4665-4671 (1985).

5. Tarentino A. L., G. Quinones, W. P. Schrader, L. M. Changchien and T. H. Plummer Jr. Multiple endoglycosidase (Endo) F activities expressed by Flavobacterium meningosepticum. Endo F1: molecular cloning, primary sequence, and structural relationship to Endo H**. J Biol Chem 267:**3868-3872 (1992).

6. Tarentino A. L., G. Quinones, L. M. Changchien, and T. H. Plummer Jr. Multiple endoglycosidase F activities expressed by Flavobacterium meningosepticum endoglycosidases F2 and F3: Molecular cloning, primary sequence, and enzyme expression. **J Biol Chem 268(13):**9702-9708 (1993).

7. Tarentino A. L. and T. H. Plummer Jr. Substrate specificity of Flavobacterium meningosepticum: Endo F2 and endo F3: purity is the name of the game. **Glycobiology 4:**771-773 (1994).

8. Tarentino, A. L. and T. H. Plummer Jr. Enzymatic deglycosylation of asparagine-linked glycans: purification, properties and specificity of oligosaccharide-cleaving enzymes from Flavobacterium meningosepticum. Methods in **Enzymology 230:**44-57 (1994).

9. Tarentino A. L., G. Quinones and T. H. Plummer Jr. Overexpression and purification of non-glycosylated recombinant endo-beta-N-acetylglucosaminidase F3**. Glycobiology 5:**599-601 (1995).

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10. Trimble, R. B. and A. L. Tarentino. Identification of Distinct Endoglycosidase (Endo) Activities in Flavobacterium meningosepticum: Endo F1, Endo F2 and Endo F3. **J. Biol Chem 266:**1646-1651 (1991).

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Figure 1 - Cleavage of oligosaccharides by Endo F2

Man - Mannose; Gal - Galactose; GlcNAc - N-acetylglucosamine; NeuAc - N-acetylneuraminic acid