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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 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 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 miricol*a.

Product Code: GE 47

Specifications

Activity: ≥ 16 U/mg, ≥ 17 U/mL

Storage: Store at 4ºC. 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 tempertures 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 37ºC 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 37ºC, 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 90ºC 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 37ºC.

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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Figure 1 - Cleavage of oligosaccharides by Endo F1 and PNGase F
Man - Mannose; GlcNAc - N-acetylglucosamine