Selectin Biosciences Inc.

Endoglycosidase H

Endoglycosidase H [Endo-β-N-acetylglucosaminidase H, EC 3.2.1.96] cleaves asparagine-linked oligomannose and hybrid, but not complex, oligosaccharides from glycoproteins (see Figure). 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. Detergent and heat denaturation may increase the rate of cleavage for some glycoproteins.

Endoglycosidase H is produced from a Streptomyces plicatus clone.

Product Code: GE 44

Specifications

Activity: 40 U/mg, ~5 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, 50 mM NaCl 1 mM EDTA.

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

Product Description

Molecular weight: 29,000 daltons

Purity: Endoglycosidase H 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 Endo H 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 PNGase buffer 5.5- 250 mM sodium phosphate pH 5.5.
- Denaturation solution- 2% w/v sodium lauryl sulfate, 1 M β-mercaptoethanol.

Suggestions for Use

Procedure for deglycosylation

1. Add up to 200 µg of glycoprotein to Eppendorf tube. Adjust to 37.5 µl final volume with deionized water.

2. Add 10 µl 5X Endoglycosidase H Buffer and 2.5 µl of Denaturation Solution (SDS/-ME). Heat at 100°C for 5 minutes.

NOTE: It is not necessary to add Triton X-100. SDS will not inactivate Endoglycosidase H.

3. Cool. Add 2.0 µl of Endoglycosidase H to the reaction. Incubate 3 hours at 37°C.

If SDS or heat denaturation is omitted, it may be necessary to increase incubation time.

References

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