Endo H Denatured Protocol for Deglycosylating Glycoproteins

Mike Gibson

Abstract

Endo H cleaves Asparagine-linked hybrid or high mannose oligosaccharides, but not complex oligosaccharides. 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.

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Materials

Endo H E-EH02 by QA-Bio Inc

Protocol

Step 1.
Add up to 200 µg of glycoprotein to an Eppendorf tube. Adjust to 37.5 µl final volume with de-ionized water.

Step 2.
Add 10 µl 5x Reaction Buffer 5.5 and 2.5 µl of Denaturation Solution. Heat at 100°C for 5 minutes.(NOTE: It is not necessary to add Triton X-100. SDS will not inactivate Endo H.)

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

Step 4.
Monitor cleavage by SDS-PAGE.