Endo H Denatured Protocol for Deglycosylating Glycoproteins

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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.

MATERIALS

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We use this protocol and it's working

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

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.)

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

4. Monitor cleavage by SDS-PAGE.