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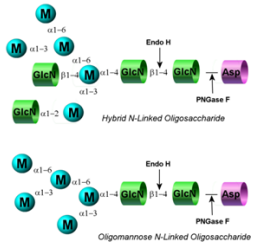
Endo H Denatured Protocol for Deglycosylating Glycoproteins



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Mike Gibson[1]

[1]QA-Bio Inc

Mike Gibson

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External link: http://www.qa-bio.com/docs/E-EH02.QA-Bio.specsheet.pdf

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Protocol status: Working
We use this protocol and it's working

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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

MATERIALS

Endo H **QA-Bio Inc** **Catalog #**E-EH02

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