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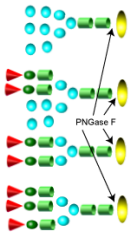
QA-Bio PNGase F Denatured Glycoprotein Protocol



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External link: https://www.qa-bio.com/product/pngase-f/

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

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Keywords: PNGase F, oligosaccharides from glycoprotein, oligosaccharide, plant glycoprotein, deglycosylation, glycoprotein, high mannose glycan, truncated sugar molecule, alternative enzyme, glycan, enzyme, number of alternative enzyme, keeping protein, most native protein, proteins in solution, aspartic acid, charged sugar

Abstract

PNGase F cleaves N-linked (asparagine-linked) oligosaccharides from glycoproteins. The enzyme deaminates asparagine to aspartic acid, leaving the oligosaccharides intact. Denaturation increases the rate of cleavage. Most native proteins can still be completely N-deglycosylated but incubation time must be increased. The enzyme will remain fully active under reaction conditions (37˚C) for at least 96 hours. PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A.

There are a number of alternative enzymes which can be used to remove N-glycans, most especially the Endo F family of enzymes and Endo H. These enzymes cleave between the two N-acetylglucosamine residues in the core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. This leaves a charged sugar which can assist in keeping proteins in solution that precipitate after deglycosylation with PNGase F which removes the oligosaccharide intact. Endo F1 cleaves high mannose and some hybrid type N-glycans. Endo F2 will removes biantennary and high mannose (at a 40X reduced rate). Endo F3 releases of triantennarry and fucosylated biantennary N-glycans. Endo H removes hybrid or high mannose glycans.

Attachments

PDF

QA-Bio.E-PNG01.specs...

271KB

Materials

MATERIALS

PNGase F **QA-Bio Inc Catalog #**E-PNG01

1 Add up to 200 µg of glycoprotein to an Eppendorf tube.

2 Adjust to 35 µl final volume with de-ionized water.

3 Add 10 µl 5x Reaction Buffer 7.5 and 2.5 µl of Denaturation Solution.

4 Heat at 100°C for 5 minutes.

5 Cool.

6 Add 2.5 µl of Triton X-100 and mix.

7 Add 2.0 µl of enzyme to the reaction.

8 Incubate 3 hours at 37°C.