QA-Bio PNGase F Denatured Glycoprotein Protocol

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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.

Citation: Mike Gibson QA-Bio PNGase F Denatured Glycoprotein Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.sd6ea9e
Published: 06 Aug 2018

Materials

PNGase F E-PNG01 by QA-Bio Inc

Protocol

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

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

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

Step 4.
Heat at 100°C for 5 minutes.

Step 5.
Cool.

Step 6.
Add 2.5 µl of Triton X-100 and mix.

Step 7.
Add 2.0 µl of enzyme to the reaction.

Step 8.
Incubate 3 hours at 37°C.