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 We use this protocol and it's working

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Mike Gibson¹

¹QA-Bio Inc

Mike Gibson

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 remove biantennary and high mannose (at a 40X reduced rate). Endo F3 releases of triantennary and fucosylated biantennary N-glycans. Endo H removes hybrid or high mannose glycans.

ATTACHMENTS

[QA-Bio.E-PNG01.specsheet.pdf](#)

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.