QA-Bio PNGase F Non-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.

Citation: Mike Gibson QA-Bio PNGase F Non-denatured Glycoprotein Protocol. protocols.io

dx.doi.org/10.17504/protocols.io.seaebae

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 40 µl final volume with de-ionized water.

Step 3.

Add 10 µl 5x Reaction Buffer 7.5

Step 4.

Add 2.0 µl of enzyme to the reaction.

Step 5.

Incubate 3-24 hours at 37°C.