Rapid PNGase F

1-800-632-7799
info@neb.com
www.neb.com

P0741S 002140815081

P0710S

50 reactions **Lot: 0011411**

Store at 4°C **Exp: 11/15**

Description: A growing number of antibodies and antibody fusions are currently used as therapeutic agents. A conserved *N*-glycan at Asn297 of the Fc region of IgG is critical for functional activity. Moreover, some antibodies have additional *N*-glycans that, together with the conserved site, affect recognition, half-life, and immune reactions.

Antibody glycosylation is heterogeneous, and variables in cell culture can increase glycan diversity. Monitoring glycosylation during production is essential to obtain the correct glycoprotein forms.

PNGase F is the most effective enzymatic method for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F digestion deaminates the aspargine residue to aspartic acid, and leaves the oligosaccharide intact, keeping it suitable for further analysis.

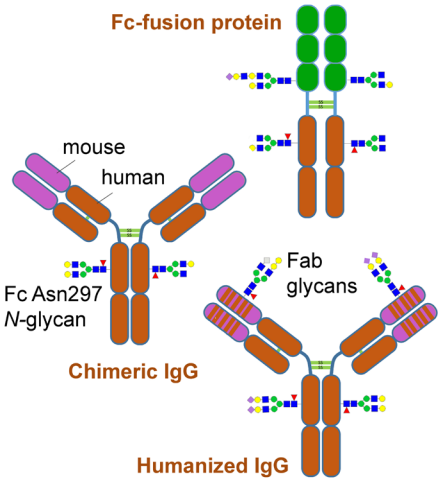


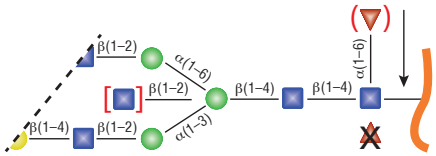
Figure 1: Possible structures of IgG and IgG-fusion proteins

Obtaining an accurate *N*-glycan profile in the shortest time possible is essential for effective process control. Typically, enzymatic release of antibody *N*-glycans using PNGase F requires an incubation time of several hours, followed by glycan derivatization and analysis by liquid chromatography and/or mass spectrometry. In addition, incomplete deglycosylation can lead to biased results. Some glycans are easier to remove than others and unless deglycosylation is extensive, the profile obtained will not represent the correct composition of the therapeutic antibody.

Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow which reduces processing time without compromising sensitivity or reproducibility.

Specificity:

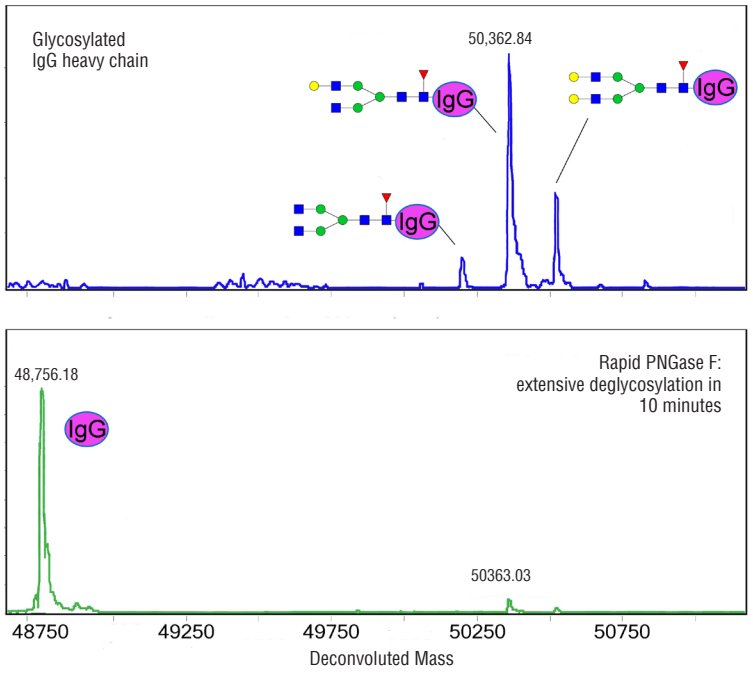
Rapid PNGase F cleaves all complex, hybrid and high-mannose type glycans from antibodies and related proteins (1). Core α1-3 fucosylation (found in immunoglobulins expressed in plant or insect cells) is resistant to both PNGase F and Rapid PNGase F.



Application:

Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in only minutes. All *N*-glycans are released rapidly and without bias, ready to be prepared for downstream chromatography or mass spectrometry analysis.

A variety of therapeutic monoclonal antibodies were used to validate Rapid PNGase F: different subclasses (IgG 1 to 4), isotypes (IgA, IgM, IgE), organisms (mouse, human, and humanized), sources (CHO, murine myeloma), and structures (IgG, IgG-fusions).



ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F.

Rapid PNGase F can effectively remove all *N*-glycans from both conserved (i.e. Fc Asn297) and non-conserved (i.e. Fab *N*-glycans) glycosylation sites. Validation was in accordance with published data. Sensitivity and specificity are not compromised by a faster and more convenient glycoprotein characterization workflow using Rapid PNGase F.

Kit Components:

Rapid PNGase F	50 µl
Rapid PNGase F Buffer (5X)	200 µl

Rapid PNGase F supplied in: 20 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM NaCl and 5 mM EDTA.

Quality Assurance: No contaminating exoglycosidase or Endoglycosidase F1, F2, or F3 activity could be detected. No contaminating proteolytic activity could be detected.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Heat Inactivation: A 20 µl reaction mixture containing 1µl of Rapid PNGase F is inactivated by incubation at 75°C for 10 minutes.

Reaction Protocols

The quantity of enzyme recommended is sufficient for the deglycosylation of 100 µg of antibody. The optimal amount of starting material will be determined by the nature of a sample (glycan diversity) and the particular downstream *N*-glycan analysis that will be performed.

Typically, 30-50 µg of antibody are sufficient for complete *N*-glycan quantitation (after labeling by reductive amination) by HILIC followed by ESI-MS. For proteins with additional glycan sites, and/or a high glycosylation variability, more starting material may be required to quantitate all minor glycan species. Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and/or larger reaction volumes. For all antibody samples tested, complete deglycosylation was achieved in 10 minutes or less. However, a given antibody may require a longer incubation time.

(see other side)

CERTIFICATE OF ANALYSIS

Typical Reaction Conditions are as follows:

For optimal heat transfer, use 0.2 ml thin wall microtubes or alternatively, 1.5 ml centrifuge tubes. A thermal cycler with heated lid, or a microtube heat block, are suitable for incubation.

One-step Protocol:

1. Combine up to 100 µg of antibody and H_2O to a volume of 16 µl.
2. Add 4 µl of Rapid PNGase F Buffer (5X) to make a 20 µl total reaction volume.
3. Add 1 µl of Rapid PNGase F.
4. Incubate 10 minutes at 50°C.
5. Prepare *N*-glycans for derivatization (i.e. reductive amination) for downstream analysis. To prepare a deglycosylated protein for mass spectrometry analysis, exchange the buffer by micro dialysis or micro filtration. Refer to www.neb.com for more detail.

Two-step Protocol:

Some antibodies (i.e. Fab N-glycans) require a pre-heating step for efficient deglycosylation.

1. Combine up to 100 µg of antibody and H_2O to a volume of 16 µl.
2. Add 4 µl of Rapid PNGase F Buffer (5X) to make a 20 µl total reaction volume.
3. Incubate at 80°C for 2 minutes, cool down.
4. Add 1 µl of Rapid PNGase F.
5. Incubate 10 minutes at 50°C.
6. Prepare *N*-glycans for derivatization (i.e. reductive amination) for downstream analysis. To prepare a deglycosylated protein for mass spectrometry analysis, exchange the buffer by micro dialysis or micro filtration. Refer to www.neb.com for more detail.

Storage: It is recommended to store this kit at 4°C. All components will be stable for at least one year if stored correctly. Do not freeze.

Notes: The target protein should be in a solution compatible with Rapid PNGase F activity. Avoid buffers containing SDS, as it inhibits PNGase F. Common stabilizing reagents such as Tween, Triton X-100, NP-40, octyl glucoside and non-detergent sulfobetaine, as well as traces of organic solvents, can prevent optimal rapid deglycosylation.

To prepare samples for liquid chromatography and/or mass spectrometry, *N*-glycans can be isolated by conventional solid phase extraction methods such as C18 and graphitized carbon.

Although this product has been optimized for the rapid removal of *N*-glycans from antibodies, it can be utilized with various other glycoproteins.

References

1. Maley, F. et al. (1989) *Anal. Biochem.* 180, 195–204.