TECHNICAL NOTES

PNGase F, Recombinant

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Glycosidase with Faster Kinetics and Greater Activity Against Native Proteins

PNGase F is a mutant recombinant glycosidase from *Flavobacterium meningosepticum*, expressed and purified from *E. coli*. The enzyme catalyzes the cleavage of N-linked oligosaccharides between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from N-linked glycoproteins.

- Does not need a denaturing step
- Works on native glycoproteins and serum glycoproteins in only minutes at room temperature
- Leads to a more complete glycan release compared to other commercially available enzymes
- Especially designed and tested for mass spectrometry imaging of tissue samples (MALDI Imaging)

Digestion of native proteins in minutes

The proprietary changes made to PNGase F have been shown to have unique characteristics when compared to other commercially available enzymes. Most N-glycosidases work best under denaturing conditions. SERVA PNGase F, however, deglycosylates also native glycoproteins and serum glycoproteins in minutes at room temperature.

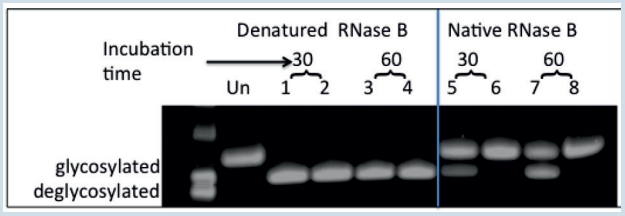

Figure 1: Coomassie stained gel of denatured or native RNase B after treatment with PNGase F.
10 µg of denatured or native RNase B were incubated with 1 µg of either SERVA PNGase F or PNGase F of another vendor for 30 min or 60 min.
Lane 1, 3, 5, 7: SERVA PNGase F
Lane 2, 4, 6, 8: Competitor PNGase F

Figure 1 shows, while both the SERVA PNGase F and the enzyme of a competitor can deglycosylate denatured RNase B in 30 minutes, only SERVA's enzyme is able to deglycosylate native RNase B in 30 minutes (lanes 5 and 7 compared to lanes 6 and 8).

More complete glycan release

The glycan analysis of the digestion products shows that digestion led to a more complete glycan release and to the cleavage of glycans not normally released by other commercially available enzymes when used at the same concentrations with the same digestion conditions. This advancement benefits applications that seek to understand glycobiology in a natural milieu.
Preliminary data indicate that SERVA PNGase F has a higher specificity towards complex (tri- and tetra-antennary) sialylated structures compared to other enzymes. Figure 2 shows a Concavalin A Lectin Blot of denatured or native human IgG after treatment with SERVA PNGase F or an enzyme of another vendor. After incubation, 10 % of the sample were separated on a SDS PAGE gel and analyzed by Lectin blotting using the lectin Concanavalin A, which binds all N-linked carbohydrates. Thus, loss of signal is taken as a measure of release of N-linked glycan.
SERVA PNGase F removes >95 % of the N-linked glycan from denatured IgG in only 2 minutes incubation, while the enzyme of the other vendor has only modest activity in the same time period (fig. 2A).

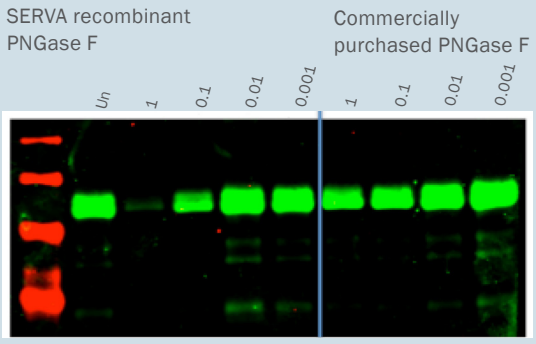

Figure 2 A: 10 µg of denatured human IgG were incubated with 1 to 0.001 µg of either SERVA PNGase F or PNGase F of another vendor for 2 min. The proteins were then separated on a SDS PAGE gel and blotted with fluorescent-labelled Concavalin A.

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In addition, as figure 2B shows, a significantly higher level of N-linked glycan was removed from native IgG, as compared to the other enzyme.

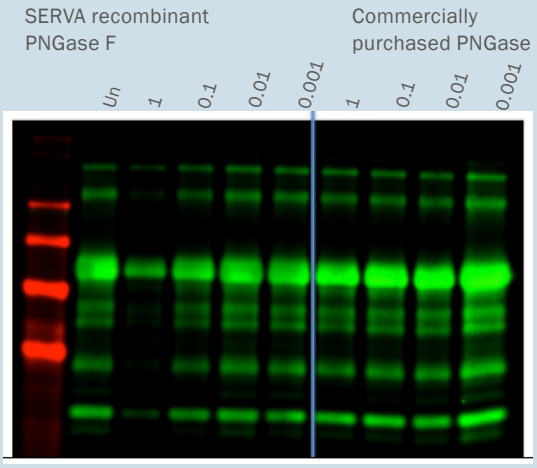

Figure 2 B: 10 µg of native human IgG were incubated with 1 to 0.001 µg of either SERVA PNGase F or PNGase F of another vendor for 1 h. The proteins were then separated on a SDS PAGE gel and blotted with fluorescent-labelled Concavalin A. Since the proteins are separated on a SDS PAGE gel, the denatured bands of IgG are present.

UPLC analysis of released N-glycans

The analysis of released N-glycans from human IgG by UPLC confirms that SERVA PNGase F removes a significantly higher level of N-linked glycans from the proteins as the enzymes from other vendors.
The chromatogram shows that with SERVA PNGase F over 3 times more sugar is released as compared to the enzyme of another vendor. Similar results were obtained with several commercially available enzymes.

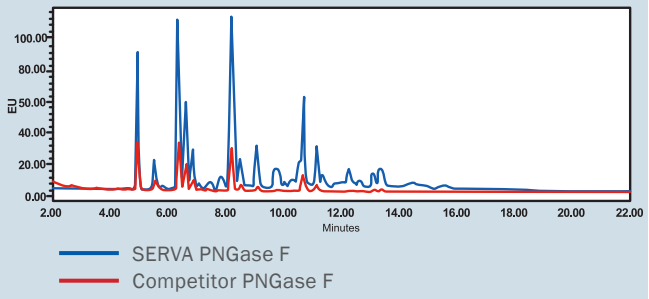

Figure 3: Chromatogram of of released N-glycans from human IgG 10 µg of human IgG were incubated with 1 µg PNGase F, labelled with a fluorescent dye and then analyzed by UPLC.

The enzyme of choice for MALDI Imaging

The faster kinetics and enhanced activity against native proteins makes it the enzyme of choice for use in MALDI Imaging.
Imaging of released glycans directly on tissue can be done following spraying of the enzyme on tissue (50,000 Units per slide) and incubation at 37 °C for 1 hour.
Since the sprayed enzyme has the potential to dry on the slide, the enzyme needs a fast kinetic. The enhanced activity against native proteins allows for the efficient deglycosylation of the proteins on the cell surface.

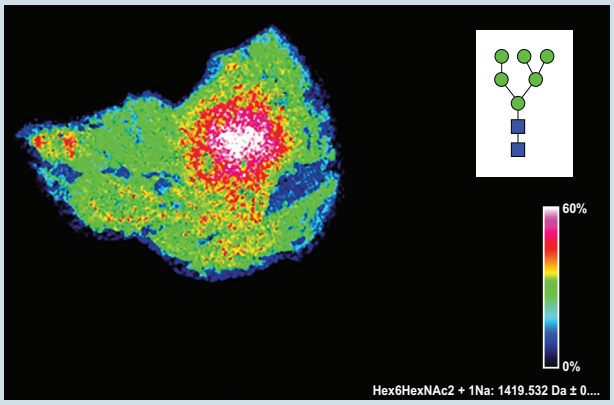

Figure 4: N-glycan image of a prostate cancer FFPE tissue

Ordering Information

Product	Size	Cat. no.
PNGase F, recombinant, 1000 U/µl (2.0 mg/ml) in 1x PBS	50 µl	36404.01
PNGase F, recombinant, lyophilized (after reconstitution 1000 U/µl, 2.0 mg/ml)	100 µg	36405.01

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