



Immobilized  
**PNGase F**



Removal of N-glycans from Glycoproteins

**SmartEnzymes™**



# Immobilized PNGase F

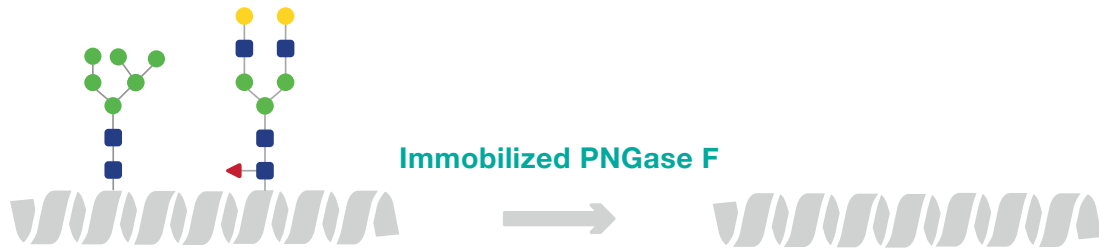
## Hydrolysis of N-glycans



Immobilized PNGase F is a resin with the PNGase F enzyme covalently coupled to agarose beads for removal of N-glycans on antibodies, fusion proteins and other N-glycosylated proteins. The enzyme is widely used for sample preparation prior to MS analysis – to reduce the protein heterogeneity and enable released glycan analysis – and to study the functional role of the N-glycan.

- N-linked glycans on glycoproteins
- Hydrolyzes the glycosidic bond between N-glycans and asparagine
- 15+ min denaturing or 1+ h native
- Native or denaturing conditions

### Deglycosylation Workflow



PNGase F (Peptide N-glycosidase F) is a glycoamidase hydrolyzing the amide bond between the polypeptide asparagine and the innermost GlcNAc of all mammalian asparagine-linked complex, hybrid, or high-mannose oligosaccharides.

During the reaction, the asparagine residue from which the glycan is removed is deamidated to aspartic acid. The glycoprotein sample is incubated with the Immobilized PNGase F resin in a spin column for 1 h to overnight using

non-denaturing conditions, or 15-60 min using denaturing conditions and the deglycosylated glycoprotein is then easily collected by a centrifugation step.

### Product Formats



#### Immobilized PNGase F

Hydrolysis of N-glycans from glycoproteins in spin columns under native conditions



#### Immobilized PNGase F Denaturing

Hydrolysis of N-glycans from glycoproteins in spin columns under denaturing conditions

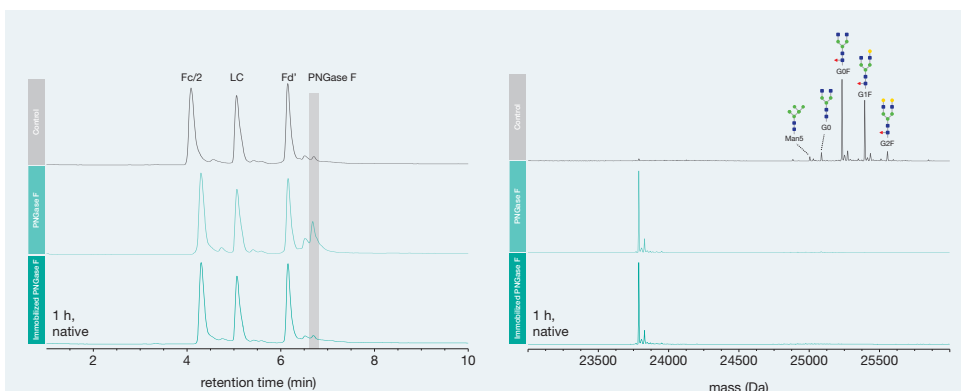
### Immobilized PNGase F

PRODUCT	DESCRIPTION	ID
Immobilized PNGase F	5 × 0.2 mg microspin columns	G1-PF6-010
Immobilized PNGase F	10 × 0.2 mg microspin columns	G1-PF6-020

## Removal of N-glycans under Native Conditions using Immobilized PNGase F



Removing the Fc N-glycan with PNGase F under native conditions enables characterization of the free N-glycan as well as the function and structure of the deglycosylated antibody. Trastuzumab was used to demonstrate the efficient removal of N-glycans by Immobilized PNGase F under native reaction conditions. The mass shift demonstrates successful removal of the Fc N-glycans with no enzyme interfering in the analysis as compared to the sample processed with PNGase F in solution.



**Removal of N-glycans under native conditions.** TIC chromatogram (left) and deconvoluted mass spectra (right) of the Fc/2 fragment of trastuzumab treated with Immobilized PNGase F or PNGase F in solution.

## Rapid N-glycan Removal using Immobilized PNGase F Denaturing

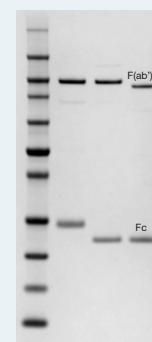
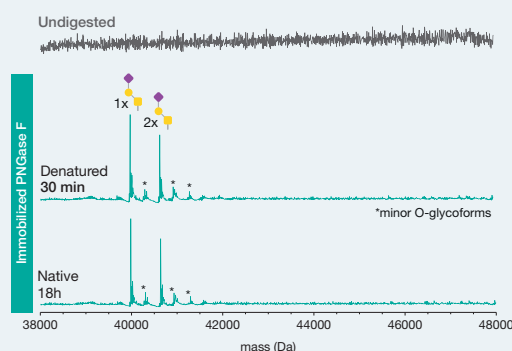


The deglycosylation ability of Immobilized PNGase F on a selection of glycoproteins is here demonstrated. For example, the

commonly used glycoprotein standard RNase B was completely deglycosylated

in 15 min using Immobilized PNGase F Denaturing.

	ABATACEPT	RNASE B	CETUXIMAB
Protein type	Fusion protein	Glycoprotein	IgG1
N-glycans	6	1	4
O-glycans	8	-	-
Reaction conditions	Native, 18 h or denaturing, 30 min	Denaturing and reducing, 15 min	Native, 1 h or denaturing, 15 min
Preparation of samples for analysis	Reduced	N/A	FabRICATOR digested



**Key characteristics and deconvoluted mass spectra or SDS-PAGE assay of a selection of glycoproteins deglycosylated using Immobilized PNGase F.** Abatacept was analyzed by reverse-phase LC-MS and RNase B and cetuximab were analyzed on SDS-PAGE. The mass shifts in the deconvoluted mass spectra or on the gels show the successful removal of N-glycans from the various glycoprotein substrates.

## Immobilized PNGase F Denaturing



PRODUCT	DESCRIPTION	ID
Immobilized PNGase F Denaturing	5 × 0.2 mg microspin columns + 5 × 1 mg RapiGest™ SF	G2-PDK-010
Immobilized PNGase F Denaturing	10 × 0.2 mg microspin columns + 10 × 1 mg RapiGest™ SF	G2-PDK-020

RapiGest™ SF Surfactant included in Immobilized PNGase F Denaturing. RapiGest™ SF Surfactant is a trademark of Waters Corporation.

#### Legal and Disclaimers

All rights reserved. Genovis products may be covered by one or more patents, trademarks and copyrights owned or controlled by Genovis AB. For more information about commercial rights, please contact the Genovis team at [licensing@genovis.com](mailto:licensing@genovis.com). Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals. All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

*RapiGest™* SF Surfactant included in Immobilized PNGaseF Denaturing. *RapiGest™* SF Surfactant is a trademark of Waters Corporation.

©2021 Genovis AB

## US & Canada

---

Genovis Inc.  
245 First Street, Suite 1800  
Cambridge, MA 02142  
USA

Customer service: 001 (617)-444-8421  
Order phone (toll free): 001 (855)-782-0084  
Order fax: 001 (858)-524-3006  
Email: [orders.us@genovis.com](mailto:orders.us@genovis.com)

## EMEA & Asia

---

Genovis AB  
Box 790  
SE-220 07 Lund  
Sweden

Customer service: +46 46 10 12 30  
Order phone: +46 46 10 12 30  
Order fax: +46 46 12 80 20  
Email: [order@genovis.com](mailto:order@genovis.com)



---

[info@genovis.com](mailto:info@genovis.com) | [www.genovis.com](http://www.genovis.com)