

Deglycosylation Topics



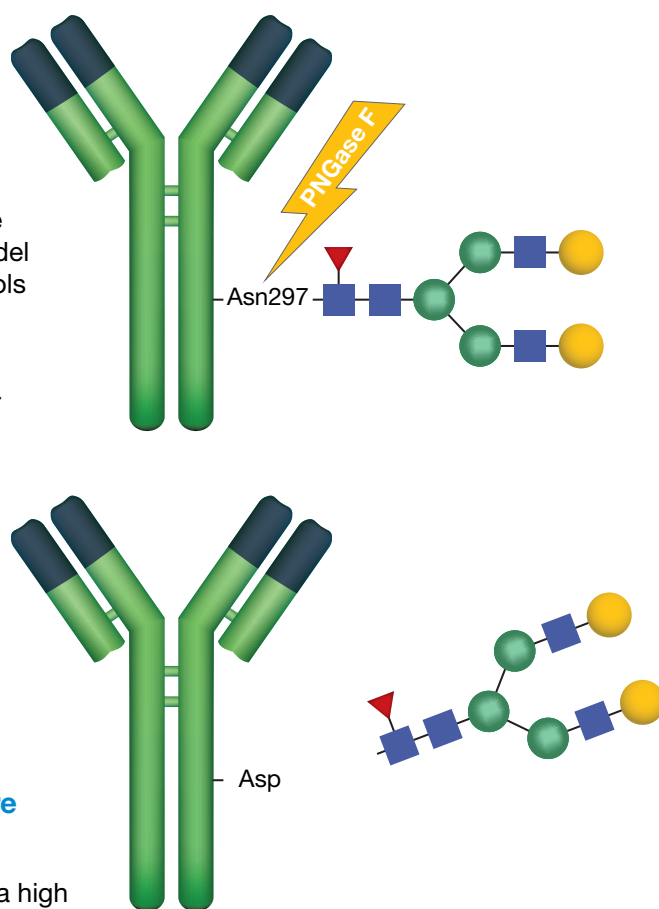
Dani Xing
Technical Guru - Bioseparations

How should I deglycosylate my antibody?

PNGase F is an endoglycosidase that cleaves N-glycans without bias, except for any that are core fucosylated $\alpha(1-3)$. If you're working with insects and plants, congrats, you're doing some rather interesting work in the world of glycobiology.

Most protocols for PNGase F were originally developed to deglycosylate complex glycoproteins (i.e. proteins with multiple glycosylation sites). For example, bovine fetuin, a common model glycoprotein, has 18 glycosylation sites.¹ As such, most protocols are developed using overnight deglycosylation to ensure deglycosylation is complete.

However, if you need answers tomorrow, what can you do? For a less complex glycoprotein like an IgG1 (2 glycosylation sites in the conserved region at Asn297), a shorter digestion time is acceptable. In fact, most vendors sell PNGase F formulated for faster deglycosylation, in some cases ten minutes or less. Further, because the glycosylation sites are easily accessible, no denaturation is required.²



Why should I deglycosylate my ADC or antibody before intact mass?

Depending on how many different glycoforms the sample has, a high degree of complexity in glycosylation could lead to extremely messy spectra, which is especially difficult with antibody-drug conjugates (ADCs).

In these cases, deglycosylation should provide a much cleaner spectra, thus providing a better assessment of relative quantitation of different drug-to-antibody ratio (DAR) species, as well as average DAR.

Always keep in mind, deglycosylation of the N-linked glycan yields an aspartic acid (ASP), resulting in a mass shift of 1 Da. Also, PNGase F reactions buffer is typically a Tris buffer (i.e. relatively high pH). Deamidation might be observed, commonly with the N-G motif; therefore faster deglycosylation protocols might be desired.

1. Nwosu, Charles C., et al. "Simultaneous and Extensive Site-Specific N- and O-Glycosylation Analysis in Protein Mixtures." *Journal of Proteome Research*, vol. 10, no. 5, June 2011, pp. 2612-2624., doi:10.1021/pr2001429

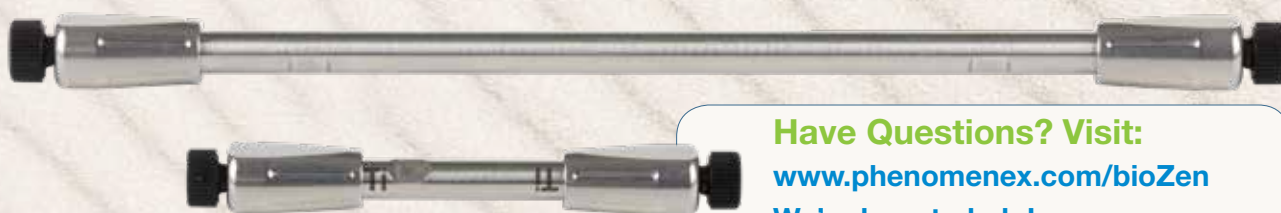
2. Hosfield, C., Engel, L., Paguio, A., Surowy, T., Jones, R., Ford, M., Urh, M., Rosenblatt, M. Recombinant PNGase F for Glycoprotein Analysis. Promega Corporation Web site. <http://www.promega.com/resources/pubhub/recombinant-pngase-f-for-glycoprotein-analysis-article/> Updated 2013. Accessed January 29, 2018.

Product Ordering Information

bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)	Phases							Biocompatible Guard Cartridges*	
	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	150 x 4.6	300 x 4.6	for 2.1 mm	for 4.6 mm
bioZen 2.6 µm Glycan	—	00D-4773-AN	00F-4773-AN	—	—	—	—	AJO-9800	—
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	—	00F-4770-AN	—	—	—	—	AJO-9803	—
bioZen 3 µm Peptide PS-C18	—	—	—	—	00B-4771-E0	00F-4771-E0	—	—	AJO-7606
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	—	00F-4774-AN	—	—	—	—	AJO-9806	—
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	—	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	—	AJO-9806	AJO-9808
bioZen 3.6 µm Intact C4	00B-4767-AN	—	00F-4767-AN	—	00B-4767-E0	00F-4767-E0	—	AJO-9809	AJO-9811
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	—	00F-4766-AN	—	00B-4766-E0	00F-4766-E0	—	AJO-9812	AJO-9814
bioZen 1.8 µm SEC-2	—	—	—	—	—	00F-4769-E0	00H-4769-E0	—	AJO-9850
bioZen 1.8 µm SEC-3	—	—	—	—	—	00F-4772-E0	00H-4772-E0	—	AJO-9851

*AJO-7606 requires guard holder KJO-4282. All other guard cartridges require guard holder AJO-9000.



Have Questions? Visit:
www.phenomenex.com/bioZen
 We're here to help!

guarantee

If bioZen columns in this technical note do not provide at least equivalent separations as compared to a competing column of the same phase, particle size, and dimensions, return the column with the comparative data within 45 days for a FULL REFUND.

Australia

t: +61 (0)2-9428-6444
 f: +61 (0)2-9428-6445
 auiinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
 f: +43 (0)1-319-1300
 anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
 t: +32 (0)2 511 8666 (Dutch)
 f: +31 (0)30-2383749
 beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
 f: +1 (310) 328-7768
 info@phenomenex.com

China

t: +86 400-606-8099
 f: +86 (0)22 2532-1033
 phen@agela.com

Denmark

t: +45 4824 8048
 f: +45 4810 6265
 nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
 f: +45 4810 6265
 nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
 f: +33 (0)1 30 09 21 11
 franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
 f: +49 (0)6021-58830-11
 anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
 f: +91 (0)40-3012 2411
 indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
 f: +44 1625-501796
 eireinfo@phenomenex.com

Italy

t: +39 051 6327511
 f: +39 051 6327555
 italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
 f: +31 (0)30-2383749
 nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
 f: 001-310-328-7768
 tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
 f: +31 (0)30-2383749
 nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
 f: +64 (0)9-4780952
 nzinfo@phenomenex.com

Norway

t: +47 810 02 005
 f: +45 4810 6265
 nordicinfo@phenomenex.com

Portugal

t: +351 221 450 488
 f: +34 91-413-2290
 ptinfo@phenomenex.com

Spain

t: +34 91-413-8613
 f: +34 91-413-2290
 espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
 f: +45 4810 6265
 nordicinfo@phenomenex.com

Switzerland

t: +41 61 692 20 20
 f: +41 61 692 20 22
 swissinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
 f: +44 (0)1625-501796
 ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
 f: +1 (310) 328-7768
 info@phenomenex.com

All other countries Corporate Office USA

t: +1 (310) 212-0555
 f: +1 (310) 328-7768
 info@phenomenex.com

Terms and Conditions

Subject to Phenomenex Standard Terms & Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

bioZen is a trademark of Phenomenex.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2018 Phenomenex, Inc. All rights reserved.

 **phenomenex**
 ...breaking with tradition™