2

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PNGase F Protocol (Denaturing Conditions) V.2

New England Biolabs[1]

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New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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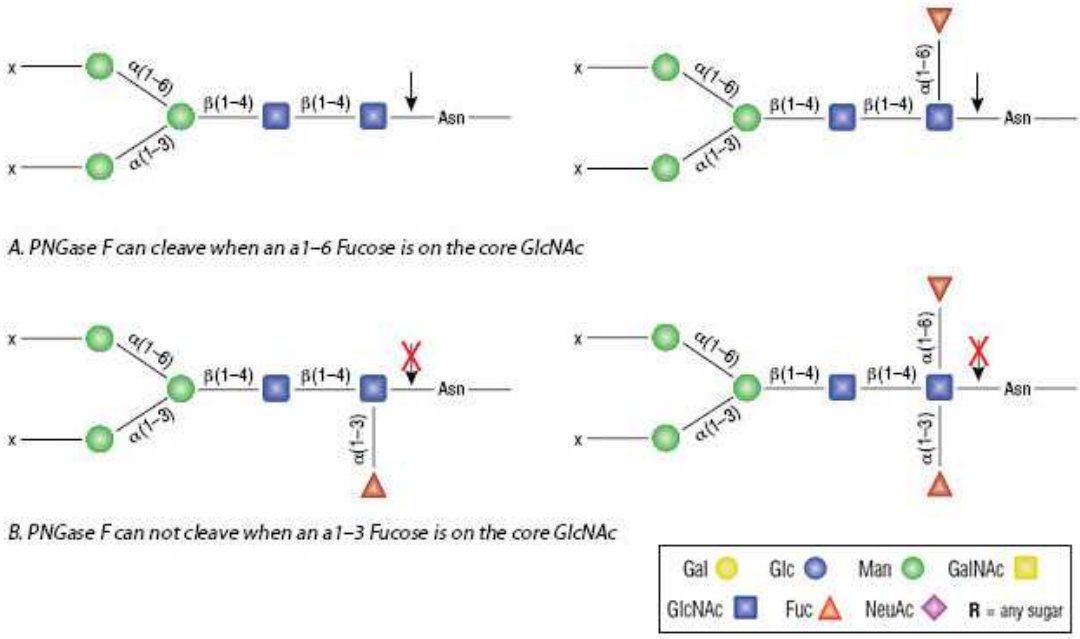
PNGase F is the most effective enzymatic method for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F is an amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides.

This is a generic PNGase F protocol for **denaturing reaction conditions**. It is appropriate for both P0704 and P0708.



A. PNGase F can cleave when an a1–6 Fucose is on the core GlcNAc

B. PNGase F can not cleave when an a1–3 Fucose is on the core GlcNAc

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https://www.neb.com/protocols/2014/07/31/pngase-f-protocol

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Liz Brydon

Essentials of Glycobiology, glygoprotein, PNGase F is inhibited, deglycosylation, PNGase F denaturing reaction conditions, PNGase F non-denaturing reaction conditions, pngase f

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- If using P0704/P0708, we recommend limiting PNGase F to 1/10 (or less) of the total reaction volume to keep the final glycerol concentration equal to (or less than) 5%.
- For unit conversion between different suppliers, please reference the Glycobiology Unit Conversion Chart page.

Biology Unit Conversion Chart

Reagent companies differ in how a unit of enzyme is defined. This chart can be used to help determine how a unit of enzyme from one company compares to a unit of enzyme from NEB. All enzymes were assayed using NEB's assay protocols as a means of normalization (NEB Assay).

Enzyme	Company	Selling Conc. (U/ml)	Units/Vial	µl/Vial	NEB Assay (U/ml)	NEB Assay Units /Vial	µl Conversion (1 NEB µl = x Company µls)
PNGase F	NEB (NEB #P0704/P0705)	500,000	15,000	30	500,000	15,000	1
	Prozyme (GKE-5006A)	2.5	0.1	40	150,000	6,000	3.3
	Prozyme (GKE-5020B, Ultra)	10	0.4	40	500,000	20,000	1
	QA Bio (E-PNG01)	5	0.3	60	200,000	12,000	2.5
	Sigma (P7367)	500	50	50	90,000	4,500	5.5

MATERIALS

PNGase F (native) - 75,000 units **New England Biolabs Catalog #P0704L**

PNGase F (native) - 15,000 units **New England Biolabs Catalog #P0704S**

PNGase F Recombinant - 75,000 units **New England Biolabs Catalog #P0708L**

PNGase F Recombinant - 15,000 units **New England Biolabs Catalog #P0708S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and larger reaction volumes. Optimal incubation times may vary for particular substrates. Typical reaction conditions are as follows:

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Denaturing Reaction Conditions:

1 

Combine **1 µg** - **20 µg glycoprotein** ,
1 µL Glycoprotein Denaturing Buffer (10X) and H_2O (if necessary) to make a **10 µL total reaction volume**.

2 

Denature glycoprotein by heating reaction at **100 °C** for **00:10:00** .

3 

Chill denatured glycoprotein **On ice** and centrifuge **00:00:10** .

4 

Make a **total reaction volume** of **20 µL** by adding: **2 µL GlycoBuffer 2 (10X)** ,
2 µL 10% NP-40 and **6 µL H2O** .

> *PNGase F is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to include NP-40 into the denaturing protocol will result in loss of enzymatic activity.*

5 

Add **1 µL PNGase F** and mix gently.

6 

Incubate reaction at **37 °C** for **01:00:00** .

7 

Analyze by method of choice.

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Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

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