PNGase F Protocol (Non-Denaturing Reaction Conditions) V.2

New England Biolabs[1]

[1]New England Biolabs

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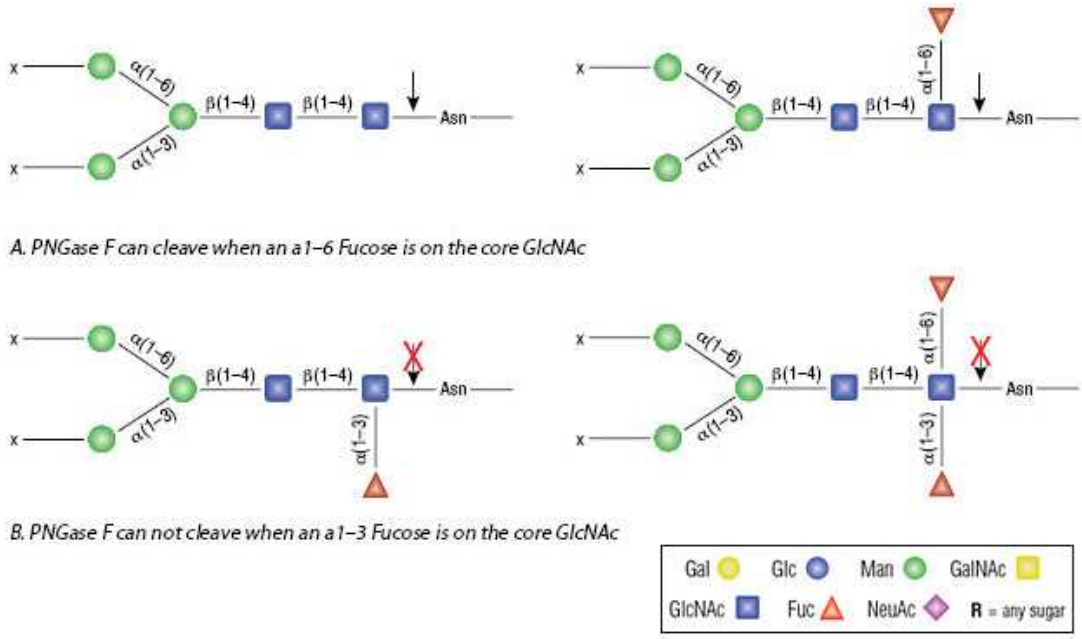
PNGase F is the most effective enzymatic method for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F is an amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides.

This is a generic PNGase F protocol for **non-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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pngasef, Essentials of Glycobiology, PNGase F is inhibited, deglycosylation, PNGase F denaturing reaction conditions, PNGase F non-denaturing reaction conditions

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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).

A	B	C	D	E	F	G	H
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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Non-Denaturing Reaction Conditions:

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When deglycosylating a native glycoprotein it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

Combine **1 µg** - **20 µg glycoprotein** , **2 µL GlycoBuffer 2 (10X)** and H_2O (if necessary) to make a **20 µL total reaction volume**.

2

Add **2 µL** - **5 µL PNGase F** , mix gently.

3

Incubate reaction at **37 °C** for **04:00:00** - **24:00:00** .

Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

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Analyze by method of choice.

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

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