For life science research only.
Not for use in diagnostic procedures.

N-Glycosidase F

Peptide-N-glycosidase F, PNGase F, peptide-N^4-(acetyl-β-glucosaminyl) asparagine amidase cloned from *Flavobacterium meningosepticum* and expressed in *E. coli*

Version: 21
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Cat. No. 11 365 169 001	100 U 0.1 ml *Not available in US*
Cat. No. 11 365 177 001	250 U 0.25 ml *Not available in US*

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	N-Glycosidase F, recombinant (*E. coli*)	Solution in 50 mM sodium phosphate, 12.5 mM EDTA, 50% glycerol (v/v), pH 7.2. *N-Glycosidase F, recombinant is also available as a lyophilizate without glycerol*.*	11 365 169 001	1 vial, 100 U (0.1 ml)
			11 365 177 001	1 vial, 250 U (0.25 ml)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	N-Glycosidase F, recombinant (*E. coli*)	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Incubation buffer for deglycosylation

- Potassium or sodium phosphate buffer
- EDTA
- Detergent, such as n-Octylglucoside*, Triton X-100*, Nonidet P-40 Substitute*, MEGA 8, or CHAPS*.
- SDS*
- 2-mercaptoethanol

The presence of mercaptoethanol has no influence on the N-glycosidase activity.

1.4. Application

N-Glycosidase F cleaves all types of asparagine bound N-glycans provided that the amino group as well as the carboxyl group are present in a peptide linkage and that the oligosaccharide has the minimum length of the chitobiose core unit.
The reaction products are ammonia, aspartic acid (in the peptide chain) and the entire oligosaccharide.

2. How to Use this Product

2.1. Before you Begin

General Considerations

The extent and rate of deglycosylation of glycoproteins depends to a high degree on the nature of the glycoprotein. Therefore, no general instructions with regard to the incubation conditions can be given.

Support of deglycosylation by detergents

In a standard protocol, most glycoproteins are, after denaturation in 1% SDS, efficiently deglycosylated in the presence of 0.5% Nonidet P-40 Substitute (SDS concentration during the N-Glycosidase F incubation: 0.1%).
If certain glycoproteins are not or not completely deglycosylated under these conditions, Nonidet P-40 Substitute can be replaced by MEGA 8 or CHAPS. These two detergents behave somewhat different in supporting the deglycosylation; therefore it should be determined in each case, which one works best for a particular sample.
The addition of a non-ionic detergent can also be beneficial in some cases for the deglycosylation of native glycoproteins.

When analyzing glycoproteins by SDS-PAGE after deglycosylation, the non-ionic detergents Triton X-100 or Nonidet P-40 Substitute will interfere with the electrophoretic separation if their concentration in the sample exceeds 1%. n-Octylglucoside and MEGA 8 cause no interferences, even at concentrations higher than 1%.

Deglycosylation of glycoproteins with N- and O-linked carbohydrate chains

Glycoproteins with N- and O-linked carbohydrate chains, for example, some serum proteins (such as erythropoietin), can be completely deglycosylated after denaturation as described by a simultaneous incubation with N-Glycosidase F, O-Glycosidase*, and Neuraminidase, for example, from *Arthrobacter ureafaciens**.
Use as buffer, 20 mM sodium phosphate, pH 7.2. 2 U N-Glycosidase F, recombinant, 2.5 mU O-Glycosidase, and 2 mU Neuraminidase are usually sufficient for the deglycosylation of 10 µg glycoprotein in a total volume of 100 µl.

Addition of protease inhibitors

N-Glycosidase F is protease-free, but protease inhibitors, such as EDTA or PMSF* can be added to inhibit proteases present in the substrate sample.

Monitoring deglycosylation

N-Glycosidase F deglycosylates a number of glycoproteins in their native form, but denaturation, such as by heating at +100°C in the presence of SDS increases the deglycosylation rate considerably.
After SDS denaturation, it is necessary to add a second detergent, such as N-Octylglucoside*, Triton X-100*, Nonidet P-40 Substitute*, octanoyl-N-methyl-glucamide (MEGA 8) or CHAPS* to the denatured sample before adding N-Glycosidase F, recombinant in order to avoid inactivation of the enzyme by SDS.

This detergent should be present in a 5- to 10-fold excess compared to the concentration of SDS.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation/Composition	For use in...
Incubation buffer	▪ 20 to 250 mM potassium or sodium phosphate buffer, pH 6 to 8.5. ▪ 10 to 50 mM EDTA, detergent, such as n-Octylglucoside*, Triton X-100*, Nonidet P40 Substitute*, MEGA 8, or CHAPS*. ▪ 0.5 to 2% sodium dodecyl sulfate (SDS)*, up to 0.2% (w/v). ▪ 2-mercaptoethanol, 1% (v/v). *2-mercaptoethanol has no influence on the N-glycosidase activity.*	Deglycosylation mixture

2.2. Protocols

Assay conditions

The required amount of enzyme depends on the actual assay conditions and should be determined for the individual cases.

3 to 300 U/ml N-Glycosidase F have been used to deglycosylate up to 0.5 mg glycoprotein/ml when incubated overnight. 0.5 mg/ml of fetuin, human transferrin, ribonuclease B, and α_1-acid glycoprotein were completely deglycosylated when using up to 300 U N-Glycosidase F in overnight incubations.

Entire deglycosylation can be monitored with the use of a glycan detection kit.

2.3. Parameters

Contaminants

Absence of contaminating activities

Contaminant	Activity
Endoglycosidase F	Not present, according to the current quality control procedures.
β-Galactosidase β-glucosidase α- and β-mannosidase β-N-acetyl-hexosaminidase α-L-fucosidase	After incubation of 100 U N-Glycosidase F, recombinant with the corresponding 10 mM 4-nitrophenyl glycosides for 17 hours at +37°C in 100 mM sodium acetate buffer, pH 5, in 0.2 ml final volume, no activity of the enzymes in question is found.
Sialidase	After incubation of 100 U N-Glycosidase F, recombinant with 6 mM N-acetyl-neuraminosyl-D-lactose for 17 hours at +37°C in 100 mM sodium acetate buffer, pH 5, in 0.2 ml final volume, no sialidase activity is found.
Proteases	After incubation of 100 U N-Glycosidase F, recombinant with 200 µg Universal Protease Substrate, Casein, resorufin-labeled* for 17 hours at +37°C in 200 µl 50 mM potassium phosphate buffer, pH 7.8, no protease activity is detected.

EC-Number

EC 3.2.218, 3.5.1.52

Molecular Weight

35.5 kDa, as calculated from the DNA sequence.

pH Optimum

pH 7.0 to 9.0.
The enzyme is also active between pH 5.0 and 7.0.

Specific Activity

Approximately 25,000 U/mg protein.

Unit Definition

One unit is the enzyme activity which hydrolyzes 1 nmol dabsyl fibrin glycopeptide or 0.2 nmol dansyl fetuin glycopeptide within 1 minute at +37°C and pH 7.8.

3. Additional Information on this Product

3.1. Test Principle

The enzyme was first detected in the endoglycosidase F preparation from the culture filtrate of *Flavobacterium meningosepticum*. The gene for N-Glycosidase F has been cloned and expressed in *E. coli* and the recombinant N-Glycosidase F purified to homogeneity.

Reaction mechanism

N-Glycosidase F cleaves all types of asparagine bound N-glycans provided that the amino group as well as the carboxyl group are present in a peptide linkage and that the oligosaccharide has the minimum length of the chitobiose core unit.
The reaction mechanism differs from that of endoglycosidases D, H, and F. These enzymes cleave the glycosidic linkage between the two N-acetylglucosamine residues. They also show a more limited substrate specificity than N-Glycosidase F. Peptide-N-glycosidase A from almond emulsion shows a similar substrate specificity as N-Glycosidase F, but is often not able to remove efficiently all susceptible oligosaccharides.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
n-Octylglucoside	10 g	10 634 425 001
CHAPS	10 g	10 810 118 001
	50 g	10 810 126 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001
Neuraminidase (Sialidase)	1 U, 100 µl	10 269 611 001
O-Glycosidase	25 mU, 50 µl	11 347 101 001
N-Glycosidase F	100 U, *Not available in US*	11 365 185 001
	250 U, *Not available in US*	11 365 193 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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