Certificate of Analysis

PNGase F

Part No.	Size
V483A	500u

Part# 9PIV483

Revised 9/13

Description: N-Glycosidase F (PNGase F) catalyzes the cleavage of N-linked oligosaccharides. PNGase F is a recombinant glycosidase cloned from *Elizabethkingia miricola*.

Biological Source: *E. coli.*

Concentration: 10,000u/ml.

Molecular Weight: PNGase F has a molecular weight of approximately 36kDa.

Physical Form: PNGase F is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 5mM EDTA at a concentration of 10,000u/ml.

Storage Conditions: Store at +2° to +10°C.

Unit Definition: One unit of PNGase F will catalyze the deglycosylation of 1 nanomole of denatured Ribonuclease B (RNase B) in one minute at 37°C. One Promega unit is equal to 1 IUB milliunit.

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Quality Control Assays

This lot passes the following Quality Control specifications:

Activity Assay: Denatured RNase B (20µg) is incubated with PNGase F for 10 minutes at 37°C, and then analyzed by SDS-PAGE. Fully glycosylated RNase B migrates at approximately 17kDa. Deglycosylation is assessed by the presence of deglycosylated RNase B with an apparent molecular weight of 13.7kDa.

Purity: ≥95% as determined by SDS-PAGE analysis.

Usage Information on Back

Signed by: J. Stevens

J. Stevens, Quality Assurance

Promega

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Usage Information

1. Protein Deglycosylation Using Recombinant PNGase F

Note: The following protocols are intended as a general guide for protein deglycosylation. Activity against different glycoprotein substrates is highly dependent on reaction conditions and should be determined empirically.

A. Protocol 1: Protein Deglycosylation Using Denaturing Conditions for SDS-PAGE

Materials to Be Supplied By the User

- 5% SDS
- 1M DTT
- 0.5M sodium phosphate buffer (pH 7.5)
- 10% NP-40

1. Add up to 50µg of the target glycoprotein in water (or a compatible buffer at a low ionic strength) to a final volume of 12µl.
2. Add 1µl 5% SDS.
3. Add 1µl of 1M DTT.
4. Denature sample by heating at 95°C for 5 minutes.
5. Cool sample for 5 minutes at room temperature.
6. Add 2µl of 0.5M sodium phosphate buffer (pH 7.5).
   Note: Other buffers can be used, if they are within the acceptable pH range for PNGase F, pH 6–10.
7. Add 2µl of 10% NP-40.
   Note: Triton® X-100 may be substituted for NP-40.
8. Add 2µl of recombinant PNGase F.
9. Incubate at 37°C for 1–3 hours.

Notes:

Deglycosylation of glycoproteins may be visualized by gel-shift on SDS-PAGE, with the deglycosylated product running faster than the glycosylated substrate.

Samples produced by SDS-PAGE are compatible with mass spectrometry analysis following standard protocols. (Please refer to the *Trypsin Gold, Mass Spectrometry Grade Technical Bulletin*, #TB309).

B. Protocol 2: Protein Deglycosylation Using Non-Denaturing Conditions for Mass Spectrometry

Materials to Be Supplied By the User

- 50mM ammonium bicarbonate buffer (pH 7.8)

1. Add up to 20µg of glycoprotein in 50mM Ammonium Bicarbonate (pH 7.8) to a final volume of 18µl.
2. Add 2µl of recombinant PNGase F.
3. Incubate at 37°C for 2–18 hours.

Notes:

Most substrates are deglycosylated more effectively when denatured. Deglycosylation under non-denaturing conditions may require increasing both the amount of PNGase F used and the incubation time. Deglycosylation of some substrates may be enhanced by the addition of up to 0.1% ProteaseMAX™ Surfactant.

Samples are compatible with downstream mass spectrometry analysis using either solution- or gel-based digestion protocols. To desalt the sample prior to MS analysis, see the ZipTip® protocol provided in the *Trypsin Gold, Mass Spectrometry Grade Technical Bulletin* #TB309.

2. References

1. *Trypsin Gold, Mass Spectrometry Grade Technical Bulletin* #TB309, Promega Corporation.
2. Mann, A.C., Self, C.H., and Turner, G.A (1994) A general method for the complete deglycosylation of a wide variety of serum glycoproteins using peptide-N-glycosidase-F. *Glycoconjugate Journal* **1**, 253–61.

3. Related Products

Product	Size	Conc.	Cat.#
Asp-N, Sequencing Grade	2µg		V1621
Arg-C, Sequencing Grade	10µg		V1881
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endo H	10,000u	500u/µl	V4871
	50,000u	500u/µl	V4875
Endoproteinase Lys-C, Sequencing Grade	5µg		V1071
Fetuin	500µg	10mg/ml	V4961
Glu-C, Sequencing Grade	50µg (5 × 10µg)		V1651
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5 × 1mg		V2072
Protein Deglycosylation Mix	20 reactions		V4931
rLys-C, Mass Spec	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073

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