

# Active Native Elizabethkingia miricola PNGase F

**Cat. No.** PNGase F-011E    **Lot. No.** (See product label)

## SPECIFICATION

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| <b>Product Overview</b> | Native Elizabethkingia miricola PNGase F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Species</b>          | Elizabethkingia miricola                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Description</b>      | <p>PNGase F cleaves N-linked (asparagine-linked) oligosaccharides from glycoproteins. The enzyme deaminates asparagine to aspartic acid, leaving the oligosaccharides intact. Denaturation increases the rate of cleavage. Most native proteins can still be completely N-deglycosylated but incubation time must be increased. The enzyme will remain fully active under reaction conditions (37 centigrade) for at least 96 hours. PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A. PNGase F cleaves N-linked (asparagine-linked) oligosaccharides from glycoproteins. The enzyme deaminates asparagine to aspartic acid, leaving the oligosaccharides intact. PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A. Denaturation increases the rate of cleavage. Most native proteins can still be completely N-deglycosylated but incubation time may need to be increased. The enzyme will remain fully active under reaction conditions (37 centigrade) for at least 96 hours. There are a number of alternative enzymes which can be used to remove N-glycans, most especially the Endo F family of enzymes and Endo H. These enzymes cleave between the two N-acetylglucosamine residues in the core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. This leaves a charged sugar which can assist in keeping proteins in solution that precipitate after deglycosylation</p> |

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|                        | with PNGase F which removes the oligosaccharide intact. Endo F1 cleaves high mannose and some hybrid type N-glycans. Endo F2 will removes biantennary and high mannose (at a 40X reduced rate). Endo F3 releases of triantennary and fucosylated biantennary N-glycans. Endo H removes hybrid or high mannose glycans.                                                                                                                                                                                                                                                                                |
| <b>Form</b>            | Sterile-filtered solution                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>EC</b>              | 3.5.1.52                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Specificity</b>     | <p>PNGase F cleaves asparagine-linked (N-linked) oligosaccharides from glycoproteins. PNGase F deaminates asparagine to aspartic acid, leaving the oligosaccharides intact. Denaturation increases the rate of cleavage up to 100×. Most native proteins can still be completely N-deglycosylated but incubation time must be increased. PNGase F will remain active under incubation conditions for at least 72 hours. PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A.</p> |
| <b>Contents</b>        | PNGase F in 20 mM Tris-HCl, pH 7.5-60μL 5× Reaction Buffer 7.5-250 mM sodium phosphate, pH 7.5 Denaturation Solution-2% SDS, 1 M Beta-mercaptoethanol Triton X-100-15% solution                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Bio-activity</b>    | Activity: 5 U/mL Specific Activity: > 25 U/mg Defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 micromole of denatured RNase B in 1 minute at 37 centigrade, pH 7.5. Cleavage is monitored by SDS-PAGE (cleaved RNase B migrates faster).                                                                                                                                                                                                                                                                                                          |
| <b>Molecular Mass</b>  | 36 kDa                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Suggested usage</b> | 1. Add up to 200 μg of glycoprotein to an Eppendorf tube. Adjust to 35 μL final                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

volume with de-ionized water. 2. Add 10  $\mu$ L 5 $\times$  Reaction Buffer 7.5 and 2.5  $\mu$ L of Denaturation Solution. Heat at 100 centigrade for 5 minutes. 3. Cool. Add 2.5  $\mu$ L of Triton X-100 and mix. 4. Add 2.0  $\mu$ L of enzyme to the reaction. Incubate 3 hours at 37 centigrade.

**Unit Definition**

One unit of PNGase F activity is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 micromole of RNase B in 1 minute at 37 centigrade, pH 7.5. Cleavage is monitored by SDS-PAGE (cleaved RNase B migrates faster).

**Purity**

PNGase F is tested for contaminating protease as follows: 10  $\mu$ g of denatured BSA is incubated at 37 centigrade for 24 hours with 2  $\mu$ L of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

**Applications**

PNGase F is suitable for the release of all types (high-mannose, hybrid and complex) N-linked glycans from glycoproteins and glycopeptides. PNGase F will not remove oligosaccharides containing  $\alpha$ (1-3) linked core fucose commonly found on plant glycoproteins.

**Stability**

Several days exposure to ambient temperatures will not reduce activity. Stable at least 24 months when stored properly.

**Storage**

Store enzyme at 4 centigrade.

**Storage Buffer**

The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl, pH 7.5

## GENE INFORMATION

**Synonyms**

PNGase F; Peptide N Glycosidase F; N-Glycosidase; N-Glycanase; png

UniProt ID

Q9XBM8

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