

# Active Recombinant Elizabethkingia miricola PNGase F

**Cat. No.** PNGase F-014E    **Lot. No.** (See product label)

## SPECIFICATION

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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| <b>Product Overview</b> | Recombinant PNGase F gene from Elizabethkingia miricola expressed in E. coli                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Species</b>          | Elizabethkingia miricola                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Source</b>           | E. coli                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Description</b>      | <p>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 <math>\alpha(1-3)</math> linked core fucose commonly found on plant glycoproteins. 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<math>\times</math>. 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 <math>\alpha(1,3)</math>-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A.</p> |
| <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<math>\times</math>. Most native proteins</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

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|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                        | <p>can still be completely N-deglycosylated but incubation time must be increased.</p> <p>PNGase F will remain active under incubation conditions for at least 72 hours.</p> <p>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>        | <p>60 µL aliquot of recombinant PNGase F (0.3 U) in 20 mM Tris-HCl, pH 7.5 5× PNGase F Reaction Buffer 7.5 for PNGase F-250 mM sodium phosphate, pH 7.5 PNGase F Denaturation Solution-2% SDS, 1 M Beta-mercaptoethanol PNGase F Triton X-100-15% solution</p>                                                                                                                          |
| <b>Bio-activity</b>    | <p>Activity: 5 U/mL Specific Activity: &gt; 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).</p>                                                                                  |
| <b>Molecular Mass</b>  | 36 kDa                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Suggested usage</b> | <p>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 µL 5× PNGase F Reaction Buffer 7.5 and 2.5 µL of PNGase F Denaturation Solution. Heat at 100 centigrade for 5 minutes. 3. Cool. Add 2.5 µL of PNGaseF Triton X-100 and mix. 4. Add 2.0 µL of PNGaseF to the reaction. Incubate 3 hours at 37 centigrade.</p> |
| <b>Unit Definition</b> | <p>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).</p>                                                                                                            |
| <b>Purity</b>          | <p>PNGase F is tested for contaminating protease as follows: 10 µg of denatured BSA is</p>                                                                                                                                                                                                                                                                                              |

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. The production host strain has been extensively tested and does not produce any detectable glycosidases.

**Stability**

Several days exposure to ambient temperatures will not reduce activity. Stable at least 12 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

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