



## Certificate of Analysis

### LudgerZyme PNGase F Release Kit

Cat. #: LZ-rPNGaseF-kit

Batch: B7CC-01

Size: 1 set of enzyme (150µL, 75 000 units) per kit

Expiry date: Oct 2019

### Product Description

This kit conforms to the specifications given in Ludger document # LZ-rPNGaseF-kit-Guide-v1.0

Each kit contains the following components:

Quantity	Cat #	Batch #	Component Name
1	LZ-PNGF-150	B7C5-03	PNGase F Enzyme Solution (Recombinant)
1	LZ-10X-REACT-01	B7C5-02	10X Reaction Buffer
1	LZ-10X-DENAT-01	B7C5-04	10X Denaturation Solution
1	LZ-NP40SOL-01	B7C5-05	NP-40 10% Solution

**Enzyme Concentration:** 500 000 units/mL

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

**Unit Definition:** One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (65 units = 1 IUB milliunit).

**Storage Temperature:** Store at +4°C

**Storage Conditions:** 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)

## LZ-rPNGaseF-kit Specification

**Contaminating Exoglycosidase and Endoglycosidase Activity Assay:** 10 µl reaction in Reaction Buffer containing 1 nM of fluorescently-labelled substrate and 5 000 units of PNGase F was incubated for 20 hours at 37°C. Activities were determined by thin layer chromatography with the following substrates (ND = not detected):

<b>Glycosidase Activity (Endo F1, F2, H)</b> Dansylated invertase high mannose	ND
<b>Glycosidase Activity (Endo F2, F3)</b> Dansylated fibrinogen biantennary	ND
<b>Glycosidase Activity (β-Mannosidase)</b> Manβ1-4Manβ1-4Man-AMC	ND
<b>Glycosidase Activity (β-Xylosidase)</b> Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC	ND
<b>Glycosidase Activity (β1-3 Galactosidase)</b> Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC	ND
<b>Glycosidase Activity (β1-4 Galactosidase)</b> Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC	ND
<b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b> GalNAcβ1-4Galβ1-4Glc-AMC	ND
<b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b> GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC	ND
<b>Glycosidase Activity (α-Glucosidase)</b> Glcα1-6Glcα1-4Glc-AMC	ND
<b>Glycosidase Activity (α-Neuraminidase)</b> Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC	ND
<b>Glycosidase Activity (α1-2 Fucosidase)</b> Fucα1-2Galβ1-4Glc-AMC	ND
<b>Glycosidase Activity (α1-3 Fucosidase)</b> Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC	ND
<b>Glycosidase Activity (α1-3 Galactosidase)</b> Galα1-3Galβ1-4GlcNAc-AMC	ND
<b>Glycosidase Activity (α1-3 Mannosidase)</b> Manα1-3Manβ1-4GlcNAc-AMC	ND
<b>Glycosidase Activity (α1-6 Galactosidase)</b> Galα1-6Galα1-6Glcα1-2Fru-AMC	ND
<b>Glycosidase Activity (α1-6 Mannosidase)</b> Manα1-6Manα1-6(Manα1-3)Man-AMC	ND
<b>Glycosidase Activity (α-N-Acetylgalactosaminidase)</b> GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC	ND

**Protease Activity Assay:** 20 µl reaction in Reaction Buffer containing 24 µg of a standard mixture of proteins and a minimum of 10 000 units of PNGase F was incubated for 20 hours at 37°C. Degradation of the protein mixture was determined by SDS-PAGE with Coomassie Blue detection (ND = not detected):

**Protease Activity**

**ND**

Standard Protein Mixture

**Protein Purity Assay:** PNGase F is  $\geq 95\%$  pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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