



Certificate of Analysis

LudgerZyme PNGase F Release Kit

Cat. #: LZ-rPNGaseF-kit

Batch: B921-01

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

Expiry date: Oct 2020

Product Description

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

Each kit contains the following components:

Quantity	Cat #	Batch #	Component Name
1	LZ-PNGF-150	B91N-06	PNGase F Enzyme Solution (Recombinant)
1	LZ-10X-REACT-01	B91N-05	10X Reaction Buffer
1	LZ-10X-DENAT-01	B91N-03	10X Denaturation Solution
1	LZ-NP40SOL-01	B91N-04	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):

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

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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