

New England Biolabs Certificate of Analysis

Product Name: PNGase F
Catalog #: P0704S/L
Concentration: 500,000 units/ml
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 NEB units = 1 IUB milliumit).
Lot #: 0431610
Assay Date: 10/2016
Expiration Date: 10/2018
Storage Temp: -20°C
Storage Conditions: 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA , 50 % Glycerol, (pH 7.5 @ 25°C)
Specification Version: PS-P0704S/L v1.0
Effective Date: 16 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0431610
Endoglycosidase F1 Activity (LC/MS) - A 20 µl reaction in Glyco Buffer 2 containing 20 pmoles of 2-AA Man-5 fluorescent standard and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent detection.	Pass
Glycosidase Activity (Endo F1, F2, H) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (Endo F2, F3) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Mannosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-Xylosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Glycosidase Activity (β1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β1-4 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β-N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-Glucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-Neuraminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 1-4GlcNAc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass

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Glycosidase Activity (α1-3 Mannosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 1-4GlcNAc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-6 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-6 Mannosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 1-3)Man-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -N-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 2 containing 24 μ g of a standard mixture of proteins and a minimum of 10,000 units of PNGase F incubated for 20 hours at 37°C, results in no detectable degradation of the protein mixture as determined by SDS-PAGE with Coomassie Blue detection.	Pass
Protein Purity Assay (SDS-PAGE) - PNGase F is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



Authorized by
Derek Robinson
16 Feb 2016



Inspected by
Alicia Bielik
19 Oct 2016

