

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	PNGase F (Glycerol-free), Recombinant
Catalog #:	P0709S/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 $\mu$ g of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l. (65 NEB units = 1 IUB milliunit).
Lot #:	0021711
Assay Date:	11/2017
Expiration Date:	11/2019
Storage Temp:	4°C
Storage Conditions:	50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)
Specification Version:	PS-P0709S/L v1.0
Effective Date:	16 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0021711
<b>Glycosidase Activity (Endo F1, F2, H)</b> - A 10 $\mu$ l 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 (Glycerol- free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (Endo F2, F3)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification (minimum release criteria)	Lot #0021711
<b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-<i>N</i>-Acetylgalactosaminidase) - A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-<i>N</i>-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</b>	Pass
<b>Glycosidase Activity (<math>\beta</math>-<i>N</i>-Acetylglucosaminidase) -</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ - <i>N</i> -Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5,000 of PNGase F (Glycerol -free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification (minimum release criteria)	Lot #0021711
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Protease Activity (SDS-PAGE)</b> - A 20 µl reaction in 1X Glyco Buffer 2 containing 24 µg of a standard mixture of proteins and a minimum of 10,000 units of PNGase F (Glycerol-free), Recombinant 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
<b>Protein Purity Assay (SDS-PAGE)</b> - PNGase F (Glycerol-free), Recombinant is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

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Authorized by Derek Robinson 16 Feb 2016



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Inspected by Brad Landgraf 14 Nov 2017