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

New England Biolabs Product Specification

Product Name: Endo F3

Catalog #: P0771S/L

Concentration: 8,000 units/ml

Unit Definition:

One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in

1 hour at 37°C in a total reaction volume of 10 μ l.

Shelf Life: 24 months
Storage Temp: -20°C

Storage Conditions: 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)

Specification Version: PS-P0771S/L v1.0
Effective Date: 28 Sep 2016

Assay Name/Specification (minimum release criteria)

Glycosidase Activity (Endo F1, F2, H) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β -Mannosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -Mannosidase substrate (Man β 1-4Man β 1-4Man-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β -Xylosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β 1-4 Galactosidase) - A 10 μ 1 reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc -AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.







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Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α -Glucosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α -Neuraminidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α 1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity ($\alpha 1$ -3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc $\alpha 1$ -3Gal $\beta 1$ -4GlcNAc $\beta 1$ -3Gal $\beta 1$ -4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity ($\alpha 1$ -3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal $\alpha 1$ -3 Gal $\beta 1$ -4 GlcNAc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity ($\alpha 1$ -3 Mannosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man $\alpha 1$ -3Man $\beta 1$ -4GlcNAc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α 1-6 Galactosidase) - A 10 μ 1 reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α 1-6 Mannosidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6(Man α 1-3)Man-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 4 containing 1 mM of fluorescently-labeled α -N -Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.







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Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 4 containing 24 μ g of a standard mixture of proteins and a minimum of 80 units of Endo F3 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.

Protein Purity Assay (SDS-PAGE) - Endo F3 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Date 28 Sep 2016

Derek Robinson
Director of Quality Control





