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## New England Biolabs Certificate of Analysis

Product Name: Endo F3
Catalog Number: P0771S
Concentration: 8,000 U/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 μl.

Packaging Lot Number: 10166668
Expiration Date: 11/2024
Storage Temperature: -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

Endo F3 Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
P0771SVIAL	Endo F3	10166667	Pass	
B1703SVIAL	10X Glycobuffer 4	10160623	Pass	

Assay Name/Specification	Lot # 10166668
Glycosidase Activity (α-Neuraminidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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.	Pass
Glycosidase Activity (β1-3 Galactosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-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.	Pass
Glycosidase Activity (β1-4 Galactosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -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.	Pass
Glycosidase Activity (α1-6 Mannosidase)	Pass



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Assay Name/Specification	Lot # 10166668
A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)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-Glucosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-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.	Pass
Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 4 containing 1 mM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-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.	Pass
Glycosidase Activity (β-Mannosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-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.	Pass
Glycosidase Activity (β-N-Acetylglucosaminidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-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.	Pass
Glycosidase Activity (β-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-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.	Pass
Glycosidase Activity (β-Xylosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-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.	Pass
Glycosidase Activity (α1-2 Fucosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled	Pass



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Assay Name/Specification	Lot # 10166668
$\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 40 units of Endo F3 incubated for 20 hours at 37 $^{\circ}$ C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity (α1-3 Fucosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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.	Pass
Glycosidase Activity (α1-3 Galactosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-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.	Pass
Glycosidase Activity (α1-3 Mannosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-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.	Pass
Glycosidase Activity (α1-6 Galactosidase) A 10 μl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-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.	Pass
Protease Activity (SDS-PAGE) A 20 µl reaction in 1X Glyco Buffer 4 containing 24 µg 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.	Pass
Protein Purity Assay (SDS-PAGE) Endo F3 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Glycosidase Activity (Endo F1, F2, H) A 10 µl 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.	Pass



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This product has been tested and shown to be in compliance with all specifications.

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Brad Landgraf Production Scientist

21 Oct 2022

Michael Tonello

Packaging Quality Control Inspector

25 Oct 2022

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