

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>Endo F2</i>
<i>Catalog #:</i>	<i>P0772S</i>
<i>Concentration:</i>	<i>8,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme required to cleave &gt;95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-P0772S v1.0</i>
<i>Effective Date:</i>	<i>11 Nov 2016</i>

### Assay Name/Specification (minimum release criteria)

**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 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**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 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**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 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**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 16 units of Endo F2 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 µl reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**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 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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**Glycosidase Activity ( $\alpha$ -Glucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -Neuraminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-2 Fucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 16 units of Endo F2 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  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 16 units of Endo F2 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  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 16 units of Endo F2 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  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-6 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-6 Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -N-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 4 containing 1 nM of fluorescently-labeled  $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 16 units of Endo F2 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Protease Activity (SDS-PAGE)** - A 20  $\mu$ l reaction in 1X Glyco Buffer 4 containing 24  $\mu$ g of a standard mixture of proteins and a minimum of 40 units of Endo F2 incubated for 20 hours at 37°C, results in no detectable degradation of the protein mixture as determined by SDS-PAGE with Coomassie Blue.



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Assay Name/Specification (minimum release criteria)
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Protein Purity Assay (SDS-PAGE) - Endo F2 is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
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Date 11 Nov 2016

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Derek Robinson  
Director of Quality Control

