

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>Bacteroides Heparinase II</i>
<b>Catalog #:</b>	P0736S/L
<b>Concentration:</b>	4,000 units/ml
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme that will liberate 1.0 $\mu$ mol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 $\mu$ l.
<b>Shelf Life:</b>	12 months
<b>Storage Temp:</b>	-80°C
<b>Storage Conditions:</b>	100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 5 mM CaCl <sub>2</sub> , (pH 7.5 @ 25°C)
<b>Specification Version:</b>	PS-P0736S/L v1.0
<b>Effective Date:</b>	09 Dec 2015

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

**Glycosidase Activity ( $\beta$ 1-3 Galactosidase)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 8 units of *Bacteroides Heparinase II* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ 1-4 Galactosidase)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 8 units of *Bacteroides Heparinase II* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -N-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 8 units of *Bacteroides Heparinase II* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 8 units of *Bacteroides Heparinase II* incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.

**Protease Activity (SDS-PAGE)** - A 20  $\mu$ l reaction in 1X Heparinase Reaction Buffer containing 24  $\mu$ g of a standard mixture of proteins and a minimum of 20 units of *Bacteroides Heparinase II* 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)** - *Bacteroides Heparinase II* is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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

**Sulfatase Activity (2-O)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled 2-O-Sulfatase substrate ( $\Delta$ UA2S-(1-4)-GlcNS6S-AMC) and 8 units of *Bacteroides* Heparinase II incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Sulfatase and Uronidase Activity (N,6-O)** - A 10  $\mu$ l reaction in Heparinase Reaction Buffer containing 1 nM of fluorescently-labeled N,6-O-Sulfatase substrate ( $\Delta$ UA-(1-4)-GlcNS6S-AMC) and 8 units of *Bacteroides* Heparinase II incubated for 20 hours at 30°C results in no detectable activity as determined by thin layer chromatography.



Date 09 Dec 2015

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Director of Quality Control

