

## New England Biolabs Product Specification

<b>Product Name:</b>	<i><math>\alpha</math>1-2,4,6 Fucosidase O</i>
<b>Catalog #:</b>	P0749S/L
<b>Concentration:</b>	2,000 units/ml
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme required to cleave >95% of the fucose from 1 nmol of G0F from human IgG [GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc(Fuc $\alpha$ 1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l.
<b>Shelf Life:</b>	12 months
<b>Storage Temp:</b>	4°C
<b>Storage Conditions:</b>	50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)
<b>Specification Version:</b>	PS-P0749S/L v1.0
<b>Effective Date:</b>	29 Aug 2017

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

**Glycosidase Activity (Endo F1, F2, H)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (Endo F2, F3)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (PNGase F)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -Xylosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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**Glycosidase Activity ( $\beta$ 1-3 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ 1-4 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

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

**Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -Glucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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

**Glycosidase Activity ( $\alpha$ 1-6 Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 4 units of  $\alpha$ 1-2,4,6 Fucosidase O 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 1 containing 24  $\mu$ g of a standard mixture of proteins and a minimum of 10 units of  $\alpha$ 1-2,4,6 Fucosidase O 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)** -  $\alpha$ 1-2,4,6 Fucosidase O is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



Date 29 Aug 2017

Derek Robinson  
Director of Quality Control

