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### New England Biolabs Product Specification

Product Name:  $\alpha 1-2$ , 3, 4, 6 Fucosidase

Catalog #: P0748S/L
Concentration: 8,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the  $\alpha$ -L-fucose from 1 nmol of Fuc  $\alpha$ 1-2Gal $\alpha$ 1-

4Glc- 7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

Shelf Life: 12 months

Storage Temp: 4°C

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

Specification Version: PS-P0748S/L v2.0
Effective Date: 23 Mar 2016

### 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.







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Glycosidase Activity ( $\beta$ -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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity ( $\beta$ 1-3 Galactosidase) - A 10  $\mu$ 1 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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  $\mu$ 1 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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  $\mu$ 1 reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\alpha$  -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 16 units of  $\alpha 1$ -2, 3, 4, 6 Fucosidase 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  $\mu$ 1 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 16 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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 1 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  $\alpha 1$ -2, 3, 4, 6 Fucosidase 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)

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 40 units of  $\alpha$ 1-2, 3, 4, 6 Fucosidase 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, 3, 4, 6 Fucosidase is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

Date 23 Mar 2016

Derek Robinson
Director of Quality Control





