

SCOVERY 240 County Road
NUINE Ipswich, MA 01938-2723

Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name: α1-2,4,6 Fucosidase O

Catalog Number: P0749L
Concentration: 2,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave >95%

of the fucose from 1 nmol of G0F from human IgG [GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)

Manβ1-4GlcNAcβ1-4GlcNAc(Fucα1-6)-AMAC], in 1 hour at 37°C in a total

reaction volume of 10 μl.

Packaging Lot Number: 10184695 Expiration Date: 03/2024 Storage Temperature: 4°C

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

Specification Version: PS-P0749S/L v1.0

α1-2,4,6 Fucosidase O Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
P0749LVIAL	α1-2,4,6 Fucosidase O	10181444	Pass	
B1727SVIAL	10X GlycoBuffer 1	10153870	Pass	

Assay Name/Specification	Lot # 10184695
Glycosidase Activity (Endo F1, F2, H) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (Endo F2, F3) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (PNGase F) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification	Lot # 10184695
<b>Glycosidase Activity (α-Glucosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at $37^{\circ}$ C results in no detectable activity as determined by thin layer chromatography.	Pass
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.	Pass
Glycosidase Activity (α-Neuraminidase) A 10 $\mu$ I reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-3 Fucosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-3 Galactosidase) A 10 $\mu$ I 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.	Pass
<b>Glycosidase Activity (α1-3 Mannosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-6 Galactosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate ( $Galα1-6Galα1-6Glcα1-2Fru-AMC$ ) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass



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Assay Name/Specification	Lot # 10184695
Glycosidase Activity (α1-6 Mannosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at $37^{\circ}$ C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (β-Mannosidase)</b> A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-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.	Pass
Glycosidase Activity (β-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 4 units of α1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 4 units of α1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 4 units of α1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 4 units of α1-2,4,6 Fucosidase O 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 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 4 units of α1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Protease Activity (SDS-PAGE)	Pass



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Assay Name/Specification	Lot # 10184695
A 20 μl reaction in 1X Glyco Buffer 1 containing 24 μg of a standard mixture of proteins and a minimum of 10 units of α1-2,4,6 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) α1-2,4,6 Fucosidase O is ≥ 95% pure as determined by SDS-PAGE analysis using	Pass

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

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Maxwell/Elkus Production Scientist 01 Mar 2023

Coomassie Blue detection.

Michael Tonello

Packaging Quality Control Inspector

20 Mar 2023



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