

## New England Biolabs Certificate of Analysis

**Product Name:**  $\alpha$ 1-2,4,6 Fucosidase O  
**Catalog Number:** P0749S  
**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 GOF from human IgG [GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc(Fuca1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.  
**Packaging Lot Number:** 10151498  
**Expiration Date:** 04/2023  
**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

<b><math>\alpha</math>1-2,4,6 Fucosidase O Component List</b>			
<b>NEB Part Number</b>	<b>Component Description</b>	<b>Lot Number</b>	<b>Individual QC Result</b>
P0749SVIAL	$\alpha$ 1-2,4,6 Fucosidase O	10144707	<b>Pass</b>
B1727SVIAL	10X GlycoBuffer 1	10121467	<b>Pass</b>

<b>Assay Name/Specification</b>	<b>Lot # 10151498</b>
<p><b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b>            A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Neuraminidase substrate (Neu5Ac<math>\alpha</math>2-3Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b>            A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc -AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b>            A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Xylosidase substrate (Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10151498
<p><b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuc<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-3Man<math>\beta</math>1-4GlcNAc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-6Gal<math>\alpha</math>1-6Glc<math>\alpha</math>1-2Fru-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10151498
<p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuc<math>\alpha</math>1-2)Gal<math>\beta</math>1-4Glc-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-6Man<math>\alpha</math>1-6(Man<math>\alpha</math>1-3)Man-AMC) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Protease Activity (SDS-PAGE)</b> A 20 <math>\mu</math>l reaction in 1X Glyco Buffer 1 containing 24 <math>\mu</math>g of a standard mixture of proteins and a minimum of 10 units of <math>\alpha</math>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.</p>	Pass
<p><b>Glycosidase Activity (Endo F2, F3)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (PNGase F)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 4 units of <math>\alpha</math>1-2,4,6 Fucosidase O incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (Endo F1, F2, H)</b></p>	Pass

Assay Name/Specification	Lot # 10151498
<p>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.</p> <p><b>Protein Purity Assay (SDS-PAGE)</b> α1-2,4,6 Fucosidase O is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<p><b>Pass</b></p>

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

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09 May 2022



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