

240 County Road 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 #: | P0749S/L |
| Concentration: | 2,000 units/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 [GlcNAc β 1-2Man α 1-6(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc(Fuc α 1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10 µl. |
| <i>Lot</i> #: | 0031801 |
| Assay Date: | 01/2018 |
| Expiration Date: | 1/2019 |
| Storage Temp: | 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 |
| Effective Date: | 29 Aug 2017 |

| Assay Name/Specification (minimum release criteria) | Lot #0031801 |
|--|--------------|
| Glycosidase Activity (Endo F1, F2, H) - A 10 μ 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 α 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 (Endo F2, F3) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 4 units of α 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 (PNGase F) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 4 units of α 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-Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled β -Mannosidase substrate (Man β 1-4Man β 1-4Man-AMC) and 4 units of α 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-Xylosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl α AMC) and 4 units of α 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 |



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| Glycosidase Activity (β1-3 Galactosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 4 units of α 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 (β1-4 Galactosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc -AMC) and 4 units of α 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-<i>N</i>-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β - <i>N</i> -Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) and 4 units of α 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-<i>N</i>-Acetylglucosaminidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β - <i>N</i> -Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 4 units of α 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-Glucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled α -Glucosidase substrate (Glc α 1-6Glc α 1-4Glc-AMC) and 4 units of α 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 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC) and 4 units of α 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 (α1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently- labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 4 units of α 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 (α1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 1-4GlcNAc-AMC) and 4 units of α 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 |



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| Assay Name/Specification (minimum release criteria) | Lot #0031801 |
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| Glycosidase Activity (α1-3 Mannosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 1-4GlcNAc-AMC) and 4 units of α 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 (α1-6 Galactosidase) - A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Gl α 1-2Fru-AMC) and 4 units of α 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 (α1-6 Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 1-3)Man-AMC) and 4 units of α 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-<i>N</i>-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α - <i>N</i> -Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC) and 4 units of α 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 |
| Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 1 containing 24 μ g of a standard mixture of proteins and a minimum of 10 units of α 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. | Pass |
| Protein Purity Assay (SDS-PAGE) - α 1-2,4,6 Fucosidase O is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |

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Authorized by Derek Robinson 29 Aug 2017



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Inspected by Alicia Bielik 26 Jan 2018