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## New England Biolabs Certificate of Analysis

Product Name: α1-3,6 Galactosidase

Catalog Number: P0731S
Concentration: 4,000 U/ml

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

of the terminal, α-D-galactose from 1 nmol

Galα1-3Galβ1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C

in a total reaction volume of 10 μl.

Packaging Lot Number: 10187114
Expiration Date: 04/2024
Storage Temperature: 4°C

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

Specification Version: PS-P0731S/L v1.0

| α1-3,6 Galactosidase Component List |                       |            |                      |  |
|-------------------------------------|-----------------------|------------|----------------------|--|
| <b>NEB Part Number</b>              | Component Description | Lot Number | Individual QC Result |  |
| P0731SVIAL                          | α1-3,6 Galactosidase  | 10183746   | Pass                 |  |
| B9001SVIAL                          | Purified BSA          | 10153875   | Pass                 |  |
| B1727SVIAL                          | 10X GlycoBuffer 1     | 10181128   | Pass                 |  |

| Assay Name/Specification  | Lot # 10187114 |
|---|----------------|
| 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 # 10187114 |  |
|---|----------------|--|
| Glycosidase Activity (α-Glucosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 20 units of α1-3, 6 Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.   | Pass           |  |
| Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC) and 20 units of α1-3, 6 Galactosidase 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 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 20 units of α1-3, 6 Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                 | Pass           |  |
| Glycosidase Activity (α1-2 Fucosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 20 units of α1-3, 6 Galactosidase 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 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 20 units of α1-3, 6 Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                       | Pass           |  |
| Glycosidase Activity (α1-3 Mannosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 20 units of α1-3, 6 Galactosidase 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 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 20 units of α1-3, 6 Galactosidase 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 # 10187114 |
|--|----------------|
| Glycosidase Activity (β-Mannosidase) A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 20 units of α1-3, 6 Galactosidase 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 μl reaction in 1X Glyco Buffer 1 containing 24 μg of a standard mixture of proteins and a minimum of 40 units of α1-3, 6 Galactosidase 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)  | Pass           |



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| Assay Name/Specification   | Lot # 10187114 |
|--|----------------|
| α1-3, 6 Galactosidase is ≥ 95% pure as determined by SDS-PAGE analysis using |                |
| Coomassie Blue detection.  |                |

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

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Maxwell/Elkus Production Scientist 31 Mar 2023 Michael Tonello

Packaging Quality Control Inspector

20 Apr 2023



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