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New England Biolabs Certificate of Analysis

Product Name: Endoglycoceramidase I (EGCase I)

Catalog Number: P0773S
Concentration: 6 U/ml

Unit Definition: One unit of R. triatomea EGCase I is defined as the amount of enzyme

required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Lot Number: 10052274
Expiration Date: 12/2020
Storage Temperature: -20°C

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

Specification Version: PS-P0773S v1.0

| Endoglycoceramidase I (EGCase I) Component List | | | | |
|---|----------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| P0773SVIAL | Endoglycoceramidase I (EGCase I) | 10031021 | Pass | |
| B0773SVIAL | EGCase I Buffer | 10031041 | Pass | |

| Assay Name/Specification | Lot # 10052274 |
|--|----------------|
| Glycosidase Activity (β1-4 Galactosidase) A 10 μl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 24 µg of a standard mixture of | Pass |



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|---|----------------|
| proteins and a minimum of 30 mU of EGCase I 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) EGCase I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| Glycosidase Activity (α1-2 Fucosidase) A 10 μl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 6 mU of EGCase I 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 μl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 6 mU of EGCase I 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 (α-N-Acetylgalactosaminidase) A 10 μl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC) and 6 mU of EGCase I 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 μl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 6 mU of EGCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity (Endo F1, F2, H) A 10 µl reaction in EGCase I Buffer containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 6 mU of EGCase I 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 EGCase I Buffer containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 6 mU of EGCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass |



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Jenna Ware Production Scientist

17 Dec 2018

Michael Tonello

Packaging Quality Control Inspector

06 Sep 2019



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