

## New England Biolabs Certificate of Analysis

**Product Name:** EGCCase I  
**Catalog #:** P0773S  
**Concentration:** 6 units/ml  
**Unit Definition:** One unit of *R. triatomea* EGCCase I is defined as the amount of enzyme required to hydrolyze 1  $\mu$ mol of ganglioside GM1a per minute at 37°C.  
**Lot #:** 0011707  
**Assay Date:** 07/2017  
**Expiration Date:** 07/2019  
**Storage Temp:** -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  
**Effective Date:** 03 Aug 2017

Assay Name/Specification (minimum release criteria)	Lot #0011707
<b>Glycosidase Activity (Endo F1, F2, H)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (Endo F2, F3)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (PNGase F)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>



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<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>

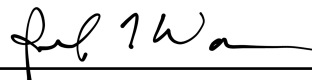


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<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - A 10 $\mu$ l reaction in EGCCase I Buffer containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 6 mU of EGCCase I incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Protease Activity (SDS-PAGE)</b> - A 20 $\mu$ l reaction in 1X EGCCase I Buffer containing 24 $\mu$ g of a standard mixture of proteins and a minimum of 30 mU of EGCCase 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - EGCCase I is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
03 Aug 2017



Inspected by  
Jenna Ware  
02 Aug 2017

