

## New England Biolabs Certificate of Analysis

**Product Name:** Endo S  
**Catalog Number:** P0741L  
**Concentration:** 200,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of native mouse monoclonal IgG in 1 hour at 37°C in a total reaction volume of 10 µl.  
**Packaging Lot Number:** 10181917  
**Expiration Date:** 03/2024  
**Storage Temperature:** 4°C  
**Storage Conditions:** 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0741S/L v2.0

Endo S Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0741LVIAL	Endo S	10179180	Pass
B1727SVIAL	10X GlycoBuffer 1	10153870	Pass

Assay Name/Specification	Lot # 10181917
<p><b>Glycosidase Activity (α1-2 Fucosidase)</b>            A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α1-3 Fucosidase)</b>            A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α1-3 Galactosidase)</b>            A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α1-3 Mannosidase)</b></p>	Pass

Assay Name/Specification	Lot # 10181917
<p>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p><b>Glycosidase Activity (α1-6 Mannosidase)</b> 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 2,000 units of Endo S 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 (α1-6 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 2,000 units of Endo S 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 (α-Glucosidase)</b> A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Endo S is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Protease Activity (SDS-PAGE)</b> A 20 µl reaction in 1X Glyco Buffer 1 containing 24 µg of a standard mixture of proteins and a minimum of 2,000 units of Endo S 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>	<b>Pass</b>
<p><b>Functional Test (Magnetic Beads, Enzyme Removal)</b> Magnetic chitin beads ( 50 µl ) were equilibrated and incubated with 2,000 units of Endo S in 300 µl of 50mM ammonium formate, pH 4.4 . The beads were pelleted using a magnetic separation rack. No Endo S was detected in the supernatant as determined by activity assay and mass spectrometry analysis.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (Endo F1, F2, H)</b> A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10181917
<p>thin layer chromatography.</p> <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(Fuca1-2)Gal<math>\beta</math>1-4Glc-AMC) and 2,000 units of Endo S 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>-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 2,000 units of Endo S 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>1-3 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 2,000 units of Endo S 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>-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 2,000 units of Endo S 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 2,000 units of Endo S 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 2,000 units of Endo S 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 2,000 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>

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

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Maxwell Elkus  
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17 Feb 2023



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22 Feb 2023