NEW ENGLAND BioLabs Inc.
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Tel 978-927-5054
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# 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:* | *10139054* |
| *Expiration Date:* | *01/2023* |
| *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 | 10137942 | **Pass** |
| B1727SVIAL | 10X GlycoBuffer 1 | 10092862 | **Pass** |

| **Assay Name/Specification** | **Lot # 10139054** |
|---|---|
| **Glycosidase Activity (α-N-Acetylgalactosaminidase)**<br>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 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α-Glucosidase)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α1-3 Galactosidase)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α1-6 Galactosidase)** | **Pass** |

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| Assay Name/Specification | Lot # 10139054 |
|---|---|
| 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 37ºC results in no detectable activity as determined by thin layer chromatography. | |
| **Glycosidase Activity (α1-6 Mannosidase)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (β-N-Acetylgalactosaminidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (β-Mannosidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (β1-3 Galactosidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α1-3 Mannosidase)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α1-3 Fucosidase)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (α1-2 Fucosidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled | **Pass** |

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| Assay Name/Specification | Lot # 10139054 |
|---|---|
| α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | |
| **Glycosidase Activity (β-Xylosidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (Endo F1, F2, H)**<br>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 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Functional Test (Magnetic Beads, Enzyme Removal)**<br>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. | **Pass** |
| **Glycosidase Activity (α-Neuraminidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Glycosidase Activity (β1-4 Galactosidase)**<br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 2,000 units of Endo S incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography. | **Pass** |
| **Protease Activity (SDS-PAGE)**<br>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. | **Pass** |
| **Protein Purity Assay (SDS-PAGE)**<br>Endo S is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |

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

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Alicia Bielik
Production Scientist
26 Jan 2022

Michael Tonello
Packaging Quality Control Inspector
26 Jan 2022