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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: 10234162
Expiration Date: 02/2025
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 v3.0

| Endo S Component List  |                       |            |                      |  |
|------------------------|-----------------------|------------|----------------------|--|
| <b>NEB Part Number</b> | Component Description | Lot Number | Individual QC Result |  |
| P0741LVIAL             | Endo S                | 10228126   | Pass                 |  |
| B1727SVIAL             | 10X GlycoBuffer 1     | 10229621   | Pass                 |  |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                                     | Lot # 10234162 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Functional Test (Magnetic Beads, Enzyme Removal) 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 (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 200 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                                          | Pass           |
| 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 200 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                                                        | Pass           |
| Glycosidase Activity (α-N-Acetylgalactosaminidase)                                                                                                                                                                                                                                                                                                           | Pass           |



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| Assay Name/Specification                                                                                                                                                                                                                                                                                                      | Lot # 10234162 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| 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 200 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                                    |                |
| 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 200 units of Endo S 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 200 units of Endo S 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 200 units of Endo S 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 Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 200 units of Endo S 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 200 units of Endo S 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 Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 200 units of Endo S 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                                                                                                                                                                                                           | Pass           |



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| Assay Name/Specification                                                                                                                                                                                                                                                                                                             | Lot # 10234162 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 200 units of Endo S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                                                                                                                                        |                |
| 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 200 units of Endo S 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 200 units of Endo S 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 200 units of Endo S 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 200 units of Endo S 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 200 units of Endo S 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 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) 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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Production Scientist

22 Feb 2024

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26 Mar 2024

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