

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

| Product Name:          | Notl  |
|------------------------|---|
| Catalog Number:        | R0189S  |
| Concentration:         | 10,000 U/ml   |
| Unit Definition:       | One unit is defined as the amount of enzyme required to digest 1 μg<br>of pBC4 DNA in NEBuffer r3.1 in 1 hour at 37°C in a total reaction<br>volume of 50 μl. |
| Packaging Lot Number:  | 10232253  |
| Expiration Date:       | 03/2026   |
| Storage Temperature:   | -20°C   |
| Storage Conditions:    | 10 mM Tris-HCl, 250 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,<br>0.15% Triton X-100, 200 μg/ml rAlbumin (pH 7.4 @ 25°C)                                   |
| Specification Version: | PS-R0189S/L/E v2.0  |

| Notl Component List |                              |            |                      |  |
|---------------------|------------------------------|------------|----------------------|--|
| NEB Part Number     | Component Description        | Lot Number | Individual QC Result |  |
| R0189SVIAL          | Notl                         | 10232248   | Pass                 |  |
| B7024AVIAL          | Gel Loading Dye, Purple (6X) | 10234873   | Pass                 |  |
| B6003SVIAL          | NEBuffer™ r3.1               | 10227733   | Pass                 |  |

| Assay Name/Specification  | Lot # 10232253 |
|---|----------------|
| Endonuclease Activity (Nicking)<br>A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a<br>minimum of 100 units of NotI incubated for 4 hours at 37°C results in <10%<br>conversion to the nicked form as determined by agarose gel electrophoresis.           | Pass           |
| Exonuclease Activity (Radioactivity Release)<br>A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and<br>double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of NotI incubated for 4<br>hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| Functional Testing (15 minute Digest)<br>A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of pBC4 DNA and 1 µl of Notl<br>incubated for 15 minutes at 37°C results in complete digestion as determined by<br>agarose gel electrophoresis.   | Pass           |
| Ligation and Recutting (Terminal Integrity)<br>After a 10-fold over-digestion of pBC4 DNA with NotI, >95% of the DNA fragments can  | Pass           |





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| Assay Name/Specification   | Lot # 10232253 |
|--|----------------|
| be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with NotI.  |                |
| Non-Specific DNase Activity (16 Hour)<br>A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of pBC4 DNA and a minimum of 100<br>units of NotI incubated for 16 hours at 37ºC results in a DNA pattern free of<br>detectable nuclease degradation as determined by agarose gel electrophoresis.   | Pass           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>NotI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue<br>detection.   | Pass           |
| <b>qPCR DNA Contamination (E. coli Genomic)</b><br>A minimum of 10 units of NotI is screened for the presence of E. coli genomic DNA<br>using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results<br>are quantified using a standard curve generated from purified E. coli genomic DNA.<br>The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome. | Pass           |

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

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YunJie Sun Production Scientist 21 Feb 2024

ton. Ma L Michae

Michael Tonello Packaging Quality Control Inspector 21 Mar 2024

