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## New England Biolabs Certificate of Analysis

| Product Name:          | Tth1111  |
|------------------------|--|
| Catalog Number:        | R0185S   |
| 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 rCutSmart Buffer in 1 hour at 65°C in a total<br>reaction volume of 50 μl. |
| Packaging Lot Number:  | 10237518   |
| Expiration Date:       | 04/2026  |
| Storage Temperature:   | -20°C  |
| Storage Conditions:    | 500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol,<br>200 μg/ml rAlbumin, (pH 7.4 @ 25°C)  |
| Specification Version: | PS-R0185S v2.0   |

| Tth111I Component List |                       |            |                      |  |
|------------------------|-----------------------|------------|----------------------|--|
| NEB Part Number        | Component Description | Lot Number | Individual QC Result |  |
| R0185SVIAL             | Tth111I               | 10237498   | Pass                 |  |
| B6004SVIAL             | rCutSmart™ Buffer     | 10233338   | Pass                 |  |

| Assay Name/Specification   | Lot # 10237518 |
|--|----------------|
| Exonuclease Activity (Radioactivity Release)<br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and<br>double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 50 units of Tth1111 incubated for<br>4 hours at 65°C releases <0.1% of the total radioactivity. | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of pBC4 DNA and 1 µl of<br>Tth111I incubated for 15 minutes at 65°C results in complete digestion as determined<br>by agarose gel electrophoresis.   | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 5-fold over-digestion of pBC4 DNA with Tth111I, ~25% of the DNA fragments<br>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments,<br>>95% can be recut with Tth111I.   | Pass           |
| Non-Specific DNase Activity (16 hour)<br>A 50 µl reaction in rCutSmart <sup>™</sup> Buffer containing 1 µg of pBC4 DNA and a minimum of<br>10 units of Tth111I incubated for 16 hours at 65°C results in a DNA pattern free of   | Pass           |





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| detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE:<br>although no nuclease degradation is detected under these conditions, extended<br>incubations and/or high concentrations of this enzyme may result in star activity.<br>See the product FAQ for recommended reaction conditions for this enzyme.   |                |
| Protein Purity Assay (SDS-PAGE)<br>Tth111I 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 Tth1111 is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA.<br>The measured level of E. coli genomic DNA contamination is $\leq$ 1 E. coli genome. | Pass           |

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

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Ana Egana Production Scientist 18 Apr 2024

Michae

Michael Tonello Packaging Quality Control Inspector 18 Apr 2024

