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New England Biolabs Certificate of Analysis

Product Name: PflMI
Catalog Number: R0509L
Concentration: 10,000 U/mI

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg

of Lambda DNA in NEBuffer r3.1 in 1 hour at 37°C in a total reaction

volume of 50 μl.

Packaging Lot Number: 10241273
Expiration Date: 03/2026
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200

μg/ml rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0509S/L v2.0

| PfIMI Component List | | | | |
|------------------------|-----------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R0509LVIAL | PfIMI | 10231510 | Pass | |
| B6003SVIAL | NEBuffer™ r3.1 | 10227734 | Pass | |

| Assay Name/Specification | Lot # 10241273 |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled pNEB193 DNA and a minimum of 50 units of PflMl incubated for 4 hours at 37°C results in <10% | Pass |
| conversion to the nicked form as determined by agarose gel electrophoresis. | Door |
| Exonuclease Activity (Radioactivity Release) A 50 μl reaction in NEBuffer [™] r3.1 containing 1 μg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of PflMI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Functional Testing (15 minute Digest) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of PflMI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 10-fold over-digestion of Lambda DNA with PflMI, ~75% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, | Pass |



R0509L / Lot: 10241273

Page 1 of 2



| Assay Name/Specification | Lot # 10241273 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| ~75% can be recut with PfIMI. | |
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 30 units of PflMI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |
| Protein Purity Assay (SDS-PAGE) PflMI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |
| qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of PflMI 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. 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.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Ana Egana
Production Scientist

13 May 2024

Josh Hersey

Packaging Quality Control Inspector

13 May 2024



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