

## New England Biolabs Certificate of Analysis

**Product Name:** *PfIMI*  
**Catalog Number:** *R0509S*  
**Concentration:** *10,000 U/ml*  
**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:** *10198130*  
**Expiration Date:** *06/2025*  
**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*

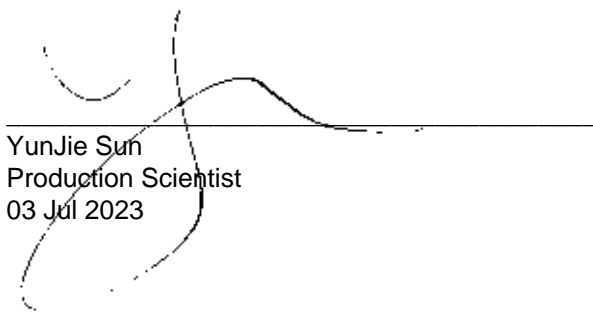
| PfIMI Component List |                       |            |                      |
|----------------------|-----------------------|------------|----------------------|
| NEB Part Number      | Component Description | Lot Number | Individual QC Result |
| R0509SVIAL           | PfIMI                 | 10196885   | Pass                 |
| B6003SVIAL           | NEBuffer™ r3.1        | 10182162   | Pass                 |

| Assay Name/Specification  | Lot # 10198130 |
|---|----------------|
| <p><b>Endonuclease Activity (Nicking)</b><br/>           A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled pNEB193 DNA and a minimum of 50 units of PfIMI incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>          | Pass           |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>           A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of PfIMI incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p> | Pass           |
| <p><b>Functional Testing (15 minute Digest)</b><br/>           A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of PfIMI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>  | Pass           |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>           After a 10-fold over-digestion of Lambda DNA with PfIMI, ~75% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments,</p>  | Pass           |

| Assay Name/Specification   | Lot # 10198130 |
|--|----------------|
| ~75% can be recut with PflMI.  |                |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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.</p>  | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>PflMI is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>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.</p> | <b>Pass</b>    |

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

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