

## New England Biolabs Certificate of Analysis

**Product Name:** PflFI  
**Catalog Number:** R0595S  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10273761  
**Expiration Date:** 12/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R0595S/L v1.0

PflFI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0595SVIAL	PflFI	10262664	Pass
B6004SVIAL	rCutSmart™ Buffer	10265039	Pass

Assay Name/Specification	Lot # 10273761
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 Units of PflFI incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 50 units of PflFI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of pBC4 DNA with PflFI, ~25% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with PflFI.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of pBC4 DNA and a minimum of 10 units of PflFI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass

Assay Name/Specification	Lot # 10273761
<b>Protein Purity Assay (SDS-PAGE)</b> PflFI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	<b>Pass</b>

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

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03 Jan 2025



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06 Jan 2025