

## New England Biolabs Certificate of Analysis

**Product Name:** PstI-HF®  
**Catalog Number:** R3140S  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10238083  
**Expiration Date:** 02/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 250 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % Triton X-100, 200 µg/ml rAlbumin, (pH 7.4 @ 25°C)  
**Specification Version:** PS-R3140S/L v3.0

PstI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3140SVIAL	PstI-HF®	10227210	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236229	Pass
B6004SVIAL	rCutSmart™ Buffer	10235560	Pass

Assay Name/Specification	Lot # 10238083
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 200 units of PstI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 200 units of PstI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of PstI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of	<b>Pass</b>

Assay Name/Specification	Lot # 10238083
<p>PstI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 100-fold over-digestion of Lambda DNA with PstI-HF®, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with PstI-HF®.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 100-fold over-digestion of Lambda DNA with PstI-HF®, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with PstI-HF®.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 200 units of PstI-HF® 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>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 200 units of PstI-HF® 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> PstI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> PstI-HF® is ≥ 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> A minimum of 20 units of PstI-HF® 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>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 20 units of PstI-HF® 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</p>	<b>Pass</b>

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genomic DNA. The measured level of E. coli genomic DNA contamination is $\leq 1$ E. coli genome.	

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](http://www.neb.com/trademarks) for additional information.



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30 Jan 2024



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03 May 2024