NEW ENGLAND BioLabs Inc.
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240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Product Name:	*SpeI-HF®*
Catalog Number:	*R3133L*
Concentration:	*20,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of pXba-XbaI DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10226568*
Expiration Date:	*01/2026*
Storage Temperature:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl , 250 mM NaCl, 0.1 mM EDTA, 50% Glycerol, 0.1% Poloxamer 188, 200 µg/ml rAlbumin, (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3133S/L/G v3.0*

SpeI-HF® Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
R3133LVIAL	SpeI-HF®	10224789	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10221467	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10219598	**Pass**

Assay Name/Specification	**Lot # 10226568**
Blue-White Screening (Terminal Integrity) A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI-HF®, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of SpeI-HF® incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 100 units of SpeI-HF® 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 rCutSmart™ Buffer containing 1 µg of pXba-XbaI digested DNA and	**Pass**

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Assay Name/Specification	Lot # 10226568
1 µl of SpeI-HF® incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of T7 DNA with SpeI-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with SpeI-HF®.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba-XbaI digested DNA and a minimum of 100 units of SpeI-HF® incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) SpeI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of SpeI-HF® is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of SpeI-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.	**Pass**

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

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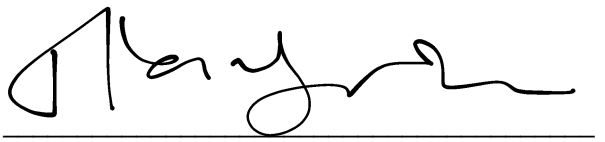
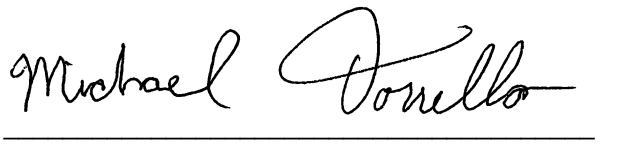


Jianying Luo
Production Scientist
10 Jan 2024



Michael Tonello
Packaging Quality Control Inspector
11 Jan 2024