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New England Biolabs Certificate of Analysis

Product Name: Spel-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-Xbal DNA in 1 hour at 37°C in a total reaction volume of 50

μl.

Packaging Lot Number: 10154631
Expiration Date: 04/2024
Storage Temperature: -20°C

Storage Conditions: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50%

Glycerol, 0.15% Triton® X-100, 200 µg/ml BSA

Specification Version: PS-R3133S/L v2.0

Spel-HF® Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R3133LVIAL	Spel-HF®	10147568	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10152092	Pass	
B6004SVIAL	rCutSmart™ Buffer	10153335	Pass	

Assay Name/Specification	Lot # 10154631
Protein Purity Assay (SDS-PAGE) Spel-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Blue-White Screening (Terminal Integrity) A sample of LITMUS28 vector linearized with a 10-fold excess of Spel-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 CutSmart® Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 60 units of Spel-HF® incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in CutSmart® Buffer containing 1 µg of pXba-Xbal digested DNA and a minimum of 100 units of Spel-HF® incubated for 16 hours at 37°C results in a DNA	Pass



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Assay Name/Specification	Lot # 10154631
pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of T7 DNA with Spel-HF®, >95% 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 Spel-HF®.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of Spel-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	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.

Penghaa Zhang Production Scientist

24 Jun 2022

Erin Varney

Packaging Quality Control Inspector

24 Jun 2022



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