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## New England Biolabs Certificate of Analysis

Product Name: Spel-HF®
Catalog Number: R3133S
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: 10164148
Expiration Date: 09/2024
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3133SVIAL	Spel-HF®	10164145	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10161525	Pass	
B6004SVIAL	rCutSmart™ Buffer	10162782	Pass	

Assay Name/Specification	Lot # 10164148
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 pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Ligation and Recutting (Terminal Integrity)	Pass



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Assay Name/Specification	Lot # 10164148
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®.	
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
Protein Purity Assay (SDS-PAGE) Spel-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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.

YunJie Sun \
Production Scientist
14 Sep 2022

Michael Tonello

Packaging Quality Control Inspector

11 Oct 2022



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