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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.

Lot Number: 10018567
Expiration Date: 06/2020
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®	10013137	Pass	
B7204SVIAL	CutSmart® Buffer	10013537	Pass	
B7024SVIAL	Gel Loading Dye, Purple (6X)	10013725	Pass	

Assay Name/Specification	Lot # 10018567
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
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
Ligation and Recutting (Terminal Integrity)  After a 20-fold over-digestion of T7 DNA with Spel-HF®, >95% of the DNA fragments	Pass



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Assay Name/Specification	Lot # 10018567
can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with Spel-HF®.	
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
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.

Tony Spear-Alfonso Production Scientist

10 Jul 2018

Michael Tonello

Packaging Quality Control Inspector

09 Aug 2018



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