

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: 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: 10034752
Expiration Date: 11/2020
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®	10027030	Pass	
B7204SVIAL	CutSmart® Buffer	10021121	Pass	
B7024SVIAL	Gel Loading Dye, Purple (6X)	10021140	Pass	

Assay Name/Specification	Lot # 10034752
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 # 10034752
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.

Anthony Francis
Production Scientist

01 Nov 2018

Michael Tonello

Packaging Quality Control Inspector

12 Feb 2019



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