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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: 10027031
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 | | | | |
|-------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R3133SVIAL | Spel-HF® | 10027030 | Pass | |
| B7204SVIAL | CutSmart® Buffer | 10021116 | Pass | |
| B7024SVIAL | Gel Loading Dye, Purple (6X) | 10021128 | Pass | |

| Assay Name/Specification | Lot # 10027031 |
|---|----------------|
| Protein Purity Assay (SDS-PAGE) Spel-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | 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) 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 |
| Blue-White Screening (Terminal Integrity) A sample of LITMUS28 vector linearized with a 10-fold excess of Spel-HF®, religated | Pass |



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|---|----------------|
| and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies. | |
| 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 |

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

Tony Spear-Alfonso **Production Scientist**

27 Sep 2018

Michael Tonello

Packaging Quality Control Inspector

02 Nov 2018



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