

*be* INSPIRED *drive* DISCOVERY *stay* GENUINE

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:        | R3133M  |
| Concentration:         | 100,000 U/ml  |
| Unit Definition:       | One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of pXba-Xbal DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l. |
| Lot Number:            | 10046390  |
| Expiration Date:       | 05/2021   |
| Storage Temperature:   | -20°C   |
| Storage Conditions:    | 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50%<br>Glycerol, 0.15% Triton® X-100, 200 μg/ml BSA                                      |
| Specification Version: | PS-R3133M v2.0  |

| Spel-HF® Component List |                              |            |                      |  |
|-------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number         | Component Description        | Lot Number | Individual QC Result |  |
| R3133MVIAL              | Spel-HF®                     | 10046389   | Pass                 |  |
| B7204SVIAL              | CutSmart® Buffer             | 10043912   | Pass                 |  |
| B7024SVIAL              | Gel Loading Dye, Purple (6X) | 10038713   | Pass                 |  |

| Assay Name/Specification   | Lot # 10046390 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI-HF®, religated<br>and transformed into an E. coli strain expressing the LacZ beta fragment gene<br>results in <1% white colonies.   | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>A 50 $\mu$ I reaction in CutSmart® Buffer containing 1 $\mu$ g of supercoiled PhiX174 DNA and<br>a minimum of 60 units of SpeI-HF® incubated for 4 hours at 37°C results in <20%<br>conversion to the nicked form as determined by agarose gel electrophoresis.  | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and<br>double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of SpeI-HF® incubated<br>for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| Ligation and Recutting (Terminal Integrity)<br>After a 20-fold over-digestion of T7 DNA with SpeI-HF®, >95% of the DNA fragments   | Pass           |





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| Assay Name/Specification   | Lot # 10046390 |
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| can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with SpeI-HF $\mbox{\sc B}$ .   |                |
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 µl reaction in CutSmart® Buffer containing 1 µg of pXba-Xbal digested DNA and a<br>minimum of 100 units of Spel-HF® incubated for 16 hours at 37°C results in a DNA<br>pattern free of detectable nuclease degradation as determined by agarose gel<br>electrophoresis. | Pass           |
| Protein Purity Assay (SDS-PAGE)<br>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.

Production Scientist 29 Apr 2019

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Jay Minichiello Packaging Quality Control Inspector 13 Jun 2019

