

## New England Biolabs Certificate of Analysis

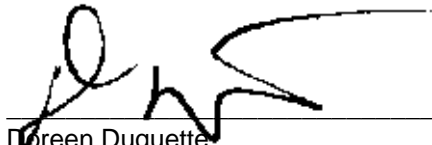
**Product Name:** Spel-HF<sup>®</sup>  
**Catalog Number:** R3133M  
**Concentration:** 100,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pXba-XbaI DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10057310  
**Expiration Date:** 10/2021  
**Storage Temperature:** -20°C  
**Storage Conditions:** 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton<sup>®</sup> X-100, 200 µg/ml BSA  
**Specification Version:** PS-R3133M v2.0

| Spel-HF <sup>®</sup> Component List |                              |            |                      |
|-------------------------------------|------------------------------|------------|----------------------|
| NEB Part Number                     | Component Description        | Lot Number | Individual QC Result |
| R3133M VIAL                         | Spel-HF <sup>®</sup>         | 10056725   | Pass                 |
| B7204S VIAL                         | CutSmart <sup>®</sup> Buffer | 10055735   | Pass                 |
| B7024S VIAL                         | Gel Loading Dye, Purple (6X) | 10046084   | Pass                 |

| Assay Name/Specification   | Lot # 10057310 |
|--|----------------|
| <b>Blue-White Screening (Terminal Integrity)</b><br>A sample of LITMUS28 vector linearized with a 10-fold excess of Spel-HF <sup>®</sup> , religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.  | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>A 50 µl reaction in CutSmart <sup>®</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of Spel-HF <sup>®</sup> incubated for 4 hours at 37°C results in <20% 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 <sup>®</sup> Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of Spel-HF <sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>After a 20-fold over-digestion of T7 DNA with Spel-HF <sup>®</sup> , >95% of the DNA fragments   | Pass           |

| Assay Name/Specification  | Lot # 10057310  |
|---|---|
| <p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with Spel-HF®.</p> <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>A 50 µl reaction in CutSmart® Buffer containing 1 µg of pXba-XbaI 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.</p> <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Spel-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p> | <p style="text-align: center;"><b>Pass</b></p> <p style="text-align: center;"><b>Pass</b></p> |

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




---

Doreen Duquette  
Production Scientist  
20 Aug 2019




---

Jay Minichiello  
Packaging Quality Control Inspector  
25 Oct 2019