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: SpeI-HF®
Catalog #: R3133M

Concentration: 100,000 units/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  $\mu$ l.

 Lot #:
 0071402

 Assay Date:
 02/2014

 Expiration Date:
 02/2016

 Storage Temp:
 -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-R3133M v1.0 Effective Date: 09 Jul 2013

Assay Name/Specification (minimum release criteria)	Lot #0071402
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI-HF <sup>TM</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
Endonuclease Activity (Nicking) - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 60 Units of SpeI-HF <sup>TM</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> - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 100 units of SpeI-HF <sup>TM</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 20-fold over-digestion of Adenovirus-2 DNA with SpeI -HF <sup>TM</sup> , >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 SpeI-HF <sup>TM</sup> .	Pass
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart <sup>TM</sup> Buffer containing 1 µg of pXba-XbaI digested DNA and a minimum of 100 units of SpeI-HF <sup>TM</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> - SpeI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass







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\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (#R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by Derek Robinson 09 Jul 2013







Inspected by Terry Petronzio 25 Feb 2014

Theren R. Returning