

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 #:	R3133S/L
Concentration:	20,000 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 μl.
<i>Lot</i> #:	0101604
Assay Date:	04/2016
Expiration Date:	04/2018
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-R3133S/L v1.0
Effective Date:	25 Apr 2016

Assay Name/Specification (minimum release criteria)	Lot #0101604
<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
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 Units of SpeI-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> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g 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
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g 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 <sup>®</sup> is $\geq$ 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.

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Authorized by Derek Robinson 25 Apr 2016



Therea R. R. Returning

Inspected by Theresa Petronzio 26 Apr 2016