

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 μl.
Lot #:	0071412
Assay Date:	12/2014
Expiration Date:	12/2016
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 #0071412
Blue-White Screening (Terminal Integrity) - A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI-HF TM , 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 [™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 Units of SpeI-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 μ l reaction in CutSmart TM Buffer containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of SpeI-HF TM incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of Adenovirus-2 DNA with SpeI -HF TM , >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 TM .	Pass
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart TM Buffer containing 1 µg of pXba- XbaI digested DNA and a minimum of 100 units of SpeI-HF TM incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Protein Purity Assay (SDS-PAGE) - SpeI-HF [®] 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 09 Jul 2013



Therea R. Retroyin

Inspected by Terry Petronzio 18 Dec 2014