NEW ENGLAND BioLabs® Inc.

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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 #:	*0151803*
Assay Date:	*03/2018*
Expiration Date:	*03/2020*
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 v2.0*
Effective Date:	*17 Jan 2018*

Assay Name/Specification (minimum release criteria)	**Lot #0151803**
Blue-White Screening (Terminal Integrity) - A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI-HF®, religated and transformed into an *E. coli* 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 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 100 units of SpeI-HF® 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 T7 DNA with SpeI-HF®, >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®.	**Pass**
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of pXba-XbaI digested DNA and a minimum of 100 units of SpeI-HF® 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 ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

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Authorized by
Derek Robinson
17 Jan 2018

Inspected by
Anthony Francis
31 Jan 2018