NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

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 rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl , 250 mM NaCl, 0.1 mM EDTA, 50% Glycerol, 0.1% Poloxamer 188, 200 µg/ml rAlbumin, (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3133M v3.0*
Effective Date:	*15 Jun 2023*

Assay Name/Specification (minimum release criteria)

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.

Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart™ 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.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in rCutSmart™ 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.

Functional Testing (15 minute Digest) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba-XbaI digested DNA and 1 µl of SpeI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

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®.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart™ 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.

Protein Purity Assay (SDS-PAGE) - SpeI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of SpeI-HF® is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of SpeI-HF® is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 15 Jun 2023

Nancy Considine
Quality Approver