

New England Biolabs Product Specification

Product Name:	<i>SphI-HF</i> [®]
Catalog #:	R3182M
Concentration:	100,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 µg of Lambda 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, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version:	PS-R3182M v2.0
Effective Date:	31 Mar 2022

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of SphI-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 SphI-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 [³H] *E. coli* DNA and a minimum of 100 units of SphI-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 Lambda DNA and 1 µl of SphI-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 Lambda DNA with SphI-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 SphI-HF[®].

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SphI-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) - SphI-HF[®] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.



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qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 20 units of SphI-HF [®] is screened for the presence of <i>E. coli</i> genomic DNA using SYBR [®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.

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Date 31 Mar 2022

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