

New England Biolabs Product Specification

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.
Shelf Life:	24 months
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 v2.0
Effective Date:	16 Jan 2018

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 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.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart[®] Buffer containing 1 µg of a mixture of single and double-stranded [³H] *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.

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 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.

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



Date 16 Jan 2018

Derek Robinson
Director of Quality Control

