

New England Biolabs Product Specification

<i>Product Name:</i>	<i>SpeI-HF[®]</i>
<i>Catalog #:</i>	<i>R3133M</i>
<i>Concentration:</i>	<i>100,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>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</i>
<i>Specification Version:</i>	<i>PS-R3133M v2.0</i>
<i>Effective Date:</i>	<i>17 Jan 2018</i>

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 17 Jan 2018

Derek Robinson
Director of Quality Control

