New England Biolabs
Product Specification

Product Name: SpeI
Catalog #: R0133S/L
Concentration: 10,000 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-R0133S/L v2.0
Effective Date: 17 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, 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 50 units of SpeI 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 50 units of SpeI 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, >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.

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 50 units of SpeI 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 is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

Derek Robinson
Director of Quality Control

Date 17 Jan 2018