Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of SphI, 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 NEBuffer 2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 Units of SphI incubated for 4 hours at 37ºC results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer 2.1 containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 100 units of SphI incubated for 4 hours at 37ºC releases <0.1% of the total radioactivity.

Ligation and Recutting (Terminal Integrity) - After a 10-fold over-digestion of Lambda DNA with SphI, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with SphI.

Non-Specific DNase Activity (16 hour) - A 50 µl reaction in NEBuffer 2.1 containing 1 µg of Lambda DNA and a minimum of 10 Units of SphI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE: although no nuclease degradation is detected under these conditions, extended incubations and/or high concentrations of this enzyme may result in star activity. See the product FAQ for recommended reaction conditions for this enzyme.

Protein Purity Assay (SDS-PAGE) - SphI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

* The BSA in this product has been granted an EDQM “Certificate of Suitability” from the European Directorate for the Quality of Medicines (R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

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Derek Robinson
Director of Quality Control

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