

New England Biolabs Product Specification

Product Name:	<i>KpnI-HF[®]</i>
Catalog #:	<i>R3142S/L</i>
Concentration:	<i>20,000 units/ml</i>
Unit Definition:	<i>One unit is defined as the amount of enzyme required to digest 1 µg of pXba DNA in 1 hour at 37°C in a total reaction volume of 50 µl.</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20 °C</i>
Storage Conditions:	<i>50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA</i>
Specification Version:	<i>PS-R3142S/L v1.0</i>
Effective Date:	<i>29 May 2013</i>

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of Litmus28i vector linearized with a 10-fold excess of KpnI-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 100 Units of KpnI-HF[™] incubated for 4 hours at 37°C results in <10% 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 200 units of KpnI-HF[™] incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Ligation and Recutting (Terminal Integrity) - After a 50-fold over-digestion of pXba DNA with KpnI-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 KpnI-HF[™].

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart[™] Buffer containing 1 µg of pXba DNA and a minimum of 100 Units of KpnI-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) - KpnI-HF[™] 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.

Date 29 May 2013

Maurice Southworth
Director of Quality Control

