

## New England Biolabs Certificate of Analysis

**Product Name:** KpnI-HF®  
**Catalog Number:** R3142S  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pXba DNA in rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10173416  
**Expiration Date:** 12/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R3142S/L/V v2.0

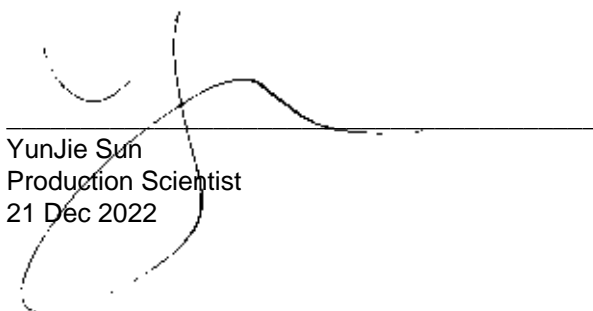
| KpnI-HF® Component List |                              |            |                      |
|-------------------------|------------------------------|------------|----------------------|
| NEB Part Number         | Component Description        | Lot Number | Individual QC Result |
| R3142SVIAL              | KpnI-HF®                     | 10173415   | Pass                 |
| B7024AVIAL              | Gel Loading Dye, Purple (6X) | 10168649   | Pass                 |
| B6004SVIAL              | rCutSmart™ Buffer            | 10173160   | Pass                 |

| Assay Name/Specification   | Lot # 10173416 |
|--|----------------|
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 µl reaction in rCutSmart™ 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. | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>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®.  | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and 1 µl of KpnI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.  | Pass           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>KpnI-HF® is >95% pure as determined by SDS PAGE analysis using Coomassie Blue  | Pass           |

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|---|----------------|
| <p>detection.</p> <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 20 units of KpnI-HF<sup>®</sup> is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is <math>\leq 1</math> E. coli genome.</p> | <b>Pass</b>    |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>A 50 <math>\mu</math>l reaction in rCutSmart<sup>™</sup> Buffer containing 1 <math>\mu</math>g of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 200 units of KpnI-HF<sup>®</sup> incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>  | <b>Pass</b>    |
| <p><b>Blue-White Screening (Terminal Integrity)</b><br/>A sample of Litmus28i vector linearized with a 10-fold excess of KpnI-HF<sup>®</sup>, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</p>   | <b>Pass</b>    |
| <p><b>Endonuclease Activity (Nicking)</b><br/>A 50 <math>\mu</math>l reaction in rCutSmart<sup>™</sup> Buffer containing 1 <math>\mu</math>g of supercoiled PhiX174 DNA and a minimum of 60 units of KpnI-HF<sup>®</sup> incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   | <b>Pass</b>    |

This product has been tested and shown to be in compliance with all specifications.

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