New England Biolabs
Product Specification

Product Name: HindIII
Catalog #: R0104T/M
Concentration: 100,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in NEBuffer r2.1 in 1 hour at 37°C in a total reaction volume of 50 µl.
Shelf Life: 24 months
Storage Temp: -20°C
Storage Conditions: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version: PS-R0104T/M v2.0
Effective Date: 08 Jul 2022

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of Litmus28i vector linearized with a 10-fold excess of HindIII, 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™ r2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of HindIII 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 NEBuffer™ r2.1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 200 units of HindIII incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and 1 µl of HindIII incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Ligation and Recutting (Terminal Integrity) - After a 200-fold over-digestion of Lambda DNA with HindIII, >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 HindIII.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda DNA and a minimum of 60 units of HindIII 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) - HindIII is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.
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<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<td>qPCR DNA Contamination (<em>E. coli Genomic</em>) - A minimum of 20 units of HindIII is screened for the presence of <em>E. coli</em> genomic DNA using SYBR® Green qPCR with primers specific for the <em>E. coli</em> 16S rRNA locus. Results are quantified using a standard curve generated from purified <em>E. coli</em> genomic DNA. The measured level of <em>E. coli</em> genomic DNA contamination is $\leq 1$ <em>E. coli</em> genome.</td>
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Derek Robinson
Director, Quality Control

Date 08 Jul 2022