NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*EagI-HF®*
Catalog #:	*R3505S/L*
Concentration:	*20,000 units/ml*
Unit Definition:	*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.*
Lot #:	*0101611*
Assay Date:	*11/2016*
Expiration Date:	*11/2018*
Storage Temp:	*-20°C*
Storage Conditions:	*500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 200 µg/ml BSA, (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3505S/L v2.0*
Effective Date:	*11 May 2016*

Assay Name/Specification (minimum release criteria)	**Lot #0101611**
Blue-White Screening (Terminal Integrity) - A sample of Litmus38i vector linearized with a 10-fold excess of EagI-HF™, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.	**Pass**
Endonuclease Activity (Nicking) - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 Units of EagI-HF™ incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 100 units of EagI-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of pXba DNA with EagI-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 EagI-HF™.	**Pass**
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 EagI-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**
Protein Purity Assay (SDS-PAGE) - EagI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	**Pass**

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* *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.*

Authorized by
Derek Robinson
11 May 2016

Inspected by
Stephanie Cornelio
22 Nov 2016