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New England Biolabs Certificate of Analysis

Product Name: EagI-HF® Catalog #: R3505M

Concentration: 100,000 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

Lot #: 0101602 Assay Date: 02/2016 Expiration Date: 02/2018 Storage Temp: -20°C

Storage Buffer: 500mM KCl, 10mM Tris-HCl (pH 7.5), 0.1mM EDTA, 1mM DTT, 500ug/ml BSA, 50% Glycerol

Specification Version: PS-R3505M v1.0 09 Jul 2015 Effective Date:

Assay Name/Specification (minimum release criteria)	Lot #0101602
Blue-White Screening (Terminal Integrity) - A sample of Litmus38i vector linearized with a 10-fold excess of EagI-HF TM , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
Endonuclease Activity (Nicking) - A 50 μl reaction in CutSmart TM Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 20 Units of EagI-HF TM 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 TM Buffer containing 1 μg of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of EagI-HF TM 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 TM, >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-HFTM.	Pass
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart TM Buffer containing 1 µg of pXba DNA and a minimum of 100 Units of EagI-HF TM 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 TM 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 09 Jul 2015







Inspected by Stephanie Cornelio 12 Feb 2016

Stephani Onetto