

## New England Biolabs Certificate of Analysis

**Product Name:** *EagI-HF*<sup>®</sup>  
**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 #:** 0081401  
**Assay Date:** 01/2014  
**Expiration Date:** 01/2016  
**Storage Temp:** -20 °C  
**Storage Conditions:** 500 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml BSA  
**Specification Version:** PS-R3505S/L v1.0  
**Effective Date:** 21 Jan 2014

| Assay Name/Specification (minimum release criteria)   | Lot #0081401 |
|---|--------------|
| <b>Blue-White Screening (Terminal Integrity)</b> - A sample of Litmus38i vector linearized with a 10-fold excess of <i>EagI-HF</i> <sup>™</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.   | <b>Pass</b>  |
| <b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 Units of <i>EagI-HF</i> <sup>™</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.                   | <b>Pass</b>  |
| <b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 100 units of <i>EagI-HF</i> <sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | <b>Pass</b>  |
| <b>Ligation and Recutting (Terminal Integrity)</b> - After a 20-fold over-digestion of pXba DNA with <i>EagI-HF</i> <sup>™</sup> , >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 <i>EagI-HF</i> <sup>™</sup> .                                      | <b>Pass</b>  |
| <b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of pXba DNA and a minimum of 100 Units of <i>EagI-HF</i> <sup>™</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.       | <b>Pass</b>  |
| <b>Protein Purity Assay (SDS-PAGE)</b> - <i>EagI-HF</i> <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.  | <b>Pass</b>  |





240 County Road  
Ipswich, MA 01938-2723

Tel 978-927-5054  
Fax 978-921-1350

www.neb.com  
info@neb.com

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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.*

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Authorized by  
Derek Robinson  
21 Jan 2014

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Inspected by  
Stephanie Doucette Cornelio  
22 Jan 2014

