

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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 $\mu$ g of pXba DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Lot #:	0051303
Assay Date:	03/2013
Expiration Date:	03/2015
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 $\mu { m g}/{ m ml}$ BSA
Specification Version:	PS-R3505S/L v1.0
Effective Date:	14 Jun 2013

Assay Name/Specification (minimum release criteria)	Lot #0051303
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of Litmus38i vector linearized with a 10-fold excess of EagI-HF <sup>TM</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in CutSmart <sup>TM</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 Units of EagI-HF <sup>TM</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 100 units of EagI-HF <sup>TM</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> - 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-HF <sup>TM</sup> .	Pass
<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 EagI-HF <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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> - EagI-HF <sup>TM</sup> 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.

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Authorized by Derek Robinson 14 Jun 2013



Stephanie Onet

Inspected by Stephanie Doucette 14 Jun 2013