

## New England Biolabs Certificate of Analysis

**Product Name:** EagI-HF<sup>®</sup>  
**Catalog Number:** R3505M  
**Concentration:** 100,000 U/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.  
**Packaging Lot Number:** 10129496  
**Expiration Date:** 12/2023  
**Storage Temperature:** -80°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-R3505M v3.0

EagI-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3505MVIAl	EagI-HF <sup>®</sup>	10129494	Pass
B7024AVIAl	Gel Loading Dye, Purple (6X)	10130600	Pass
B6004SVIAl	rCutSmart <sup>™</sup> Buffer	10132768	Pass

Assay Name/Specification	Lot # 10129496
<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>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of pXba DNA with EagI-HF <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 EagI-HF <sup>™</sup> .	Pass
<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 EagI-HF <sup>™</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> EagI-HF <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass

Assay Name/Specification	Lot # 10129496
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of EagI-HF™ incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Blue-White Screening (Terminal Integrity)</b> 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 &lt;1% white colonies.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.



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25 Jan 2022



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25 Jan 2022