

## New England Biolabs Certificate of Analysis

**Product Name:** *EagI-HF®*  
**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:** *10157686*  
**Expiration Date:** *07/2024*  
**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® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3505MVIAL	EagI-HF®	10157687	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10153339	Pass
B6004SVIAL	rCutSmart™ Buffer	10153337	Pass

Assay Name/Specification	Lot # 10157686
<b>Non-Specific DNase Activity (16 Hour)</b> 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
<b>Ligation and Recutting (Terminal Integrity)</b> 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
<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 <0.1% of the total radioactivity.	Pass
<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	Pass

Assay Name/Specification	Lot # 10157686
gene results in <1% white colonies.	
<p><b>Endonuclease Activity (Nicking)</b> 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 &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> EagI-HF™ is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>

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

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18 Jul 2022



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18 Jul 2022