

## New England Biolabs Certificate of Analysis

**Product Name:** *EagI-HF<sup>®</sup>*  
**Catalog Number:** *R3505L*  
**Concentration:** *20,000 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.*  
**Lot Number:** *10010056*  
**Expiration Date:** *06/2020*  
**Storage Temperature:** *-20°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-R3505S/L v2.0*

<b>EagI-HF<sup>®</sup> Component List</b>			
<b>NEB Part Number</b>	<b>Component Description</b>	<b>Lot Number</b>	<b>Individual QC Result</b>
R3505LVIAL	EagI-HF <sup>®</sup>	10010057	<b>Pass</b>
B7204SVIAL	CutSmart <sup>®</sup> Buffer	10013537	<b>Pass</b>
B7024SVIAL	Gel Loading Dye, Purple (6X)	10011266	<b>Pass</b>

<b>Assay Name/Specification</b>	<b>Lot # 10010056</b>
<b>Blue-White Screening (Terminal Integrity)</b> A sample of Litmus38i vector linearized with a 10-fold excess of EagI-HF <sup>™</sup> , religated and transformed into an E. coli 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 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.	<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] E. coli DNA and a minimum of 100 units of EagI-HF <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 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,	<b>Pass</b>

Assay Name/Specification	Lot # 10010056
>95% can be recut with Eagl-HF™.	
<p><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 Eagl-HF™ incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Eagl-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.



Stephanie Cornelio  
Production Scientist  
06 Jun 2018



Michael Tonello  
Packaging Quality Control Inspector  
03 Aug 2018