

## New England Biolabs Certificate of Analysis

**Product Name:** *EagI-HF<sup>®</sup>*  
**Catalog Number:** *R3505S*  
**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.*  
**Packaging Lot Number:** *10097232*  
**Expiration Date:** *01/2023*  
**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-R3505S/L v2.0*

| EagI-HF <sup>®</sup> Component List |                              |            |                      |
|-------------------------------------|------------------------------|------------|----------------------|
| NEB Part Number                     | Component Description        | Lot Number | Individual QC Result |
| R3505SVIAL                          | EagI-HF <sup>®</sup>         | 10097231   | Pass                 |
| B7204SVIAL                          | CutSmart <sup>®</sup> Buffer | 10093117   | Pass                 |
| B7024AVIAL                          | Gel Loading Dye, Purple (6X) | 10089405   | Pass                 |

| Assay Name/Specification  | Lot # 10097232 |
|---|----------------|
| <b>Non-Specific DNase Activity (16 Hour)</b><br>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><br>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>Exonuclease Activity (Radioactivity Release)</b><br>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.  | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>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%   | Pass           |

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|--|----------------|
| conversion to the nicked form as determined by agarose gel electrophoresis.  |                |
| <p><b>Blue-White Screening (Terminal Integrity)</b><br/>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>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>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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05 Feb 2021



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