

New England Biolabs Certificate of Analysis

Product Name: EagI-HF®
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.
Packaging Lot Number: 10147897
Expiration Date: 04/2024
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-R3505S/L v3.0

| EagI-HF® Component List | | | |
|-------------------------|------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| R3505LVIAL | EagI-HF® | 10147896 | Pass |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10144740 | Pass |
| B6004SVIAL | rCutSmart™ Buffer | 10146828 | Pass |

| Assay Name/Specification | Lot # 10147897 |
|--|----------------|
| Blue-White Screening (Terminal Integrity) 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 <1% white colonies. | Pass |
| Endonuclease Activity (Nicking) 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 <20% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Protein Purity Assay (SDS-PAGE) EagI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ 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 |

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|---|----------------|
| <p>Ligation and Recutting (Terminal Integrity) 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™.</p> | Pass |
| <p>Non-Specific DNase Activity (16 Hour) 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.</p> | Pass |

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 for additional information.



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Production Scientist
02 May 2022



Erin Varney
Packaging Quality Control Inspector
02 May 2022