

New England Biolabs Product Specification

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| Product Name: | <i>EagI-HF</i> [®] |
| Catalog #: | R3505S/L |
| Concentration: | 20,000 units/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. |
| Shelf Life: | 24 months |
| Storage Temp: | -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 |
| Effective Date: | 08 Feb 2021 |

Assay Name/Specification (minimum release criteria)

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.

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.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart[™] Buffer containing 1 µg of a mixture of single and double-stranded [³H] *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.

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*[™].

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.

Protein Purity Assay (SDS-PAGE) - *EagI-HF*[™] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.





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Date 08 Feb 2021

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