

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name: Eagl-HF®
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: 10163697 Expiration Date: 02/2025 Storage Temperature: -80°C

Storage Conditions: 500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol,

200  $\mu$ g/ml BSA, (pH 7.4 @ 25°C)

Specification Version: PS-R3505S/L v3.0

Eagl-HF® Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3505SVIAL	Eagl-HF®	10163695	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10173660	Pass	
B6004SVIAL	rCutSmart™ Buffer	10175290	Pass	

Assay Name/Specification	Lot # 10163697
<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
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 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.	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 Eagl-HF™ incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
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	Pass



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Assay Name/Specification	Lot # 10163697
gene results in <1% white colonies.	
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 Eagl-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Protein Purity Assay (SDS-PAGE) Eagl-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	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.

YunJie Sun \

Production Scientist 03 Feb 2023

Michael Tonello

Packaging Quality Control Inspector

06 Feb 2023



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