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## 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: 10091158
Expiration Date: 02/2022
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

Eagl-HF® Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R3505SVIAL	Eagl-HF®	10066784	Pass	
B7204SVIAL	CutSmart® Buffer	10089399	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10084973	Pass	

Assay Name/Specification	Lot # 10091158
Blue-White Screening (Terminal Integrity) A sample of Litmus38i vector linearized with a 10-fold excess of Eagl-HF™,	Pass
religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	
Protein Purity Assay (SDS-PAGE)	Pass
EagI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	
Non-Specific DNase Activity (16 Hour)	Pass
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.	
Ligation and Recutting (Terminal Integrity)	Pass
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> .	



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Assay Name/Specification	Lot # 10091158
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
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

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.

Penghaa Zhang Production Scientist

18 Nov 2020

Michael Tonello

Packaging Quality Control Inspector

18 Nov 2020



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