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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.

Lot Number: 10038776
Expiration Date: 03/2021
Storage Temperature: -20°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 v2.0

| Eagl-HF® Component List |                              |            |                      |  |
|-------------------------|------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>  | Component Description        | Lot Number | Individual QC Result |  |
| R3505SVIAL              | Eagl-HF®                     | 10038773   | Pass                 |  |
| B7204SVIAL              | CutSmart® Buffer             | 10036665   | Pass                 |  |
| B7024SVIAL              | Gel Loading Dye, Purple (6X) | 10021133   | Pass                 |  |

| Assay Name/Specification  | Lot # 10038776 |
|---|----------------|
| 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.  |                |
| Endonuclease Activity (Nicking)   | Pass           |
| 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.   |                |
| Exonuclease Activity (Radioactivity Release)  | Pass           |
| 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.                   |                |
| Ligation and Recutting (Terminal Integrity)   | Pass           |
| After a 20-fold over-digestion of pXba DNA with Eagl-HF™, >95% of the DNA fragments   |                |
| can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments,  |                |



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This product has been tested and shown to be in compliance with all specifications.

Production Scientist 15 Feb 2019 Michael Tonello

Packaging Quality Control Inspector

27 Mar 2019



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