

## New England Biolabs Certificate of Analysis

**Product Name:** NotI-HF®  
**Catalog Number:** R3189L  
**Concentration:** 20,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Lot Number:** 10017520  
**Expiration Date:** 03/2020  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R3189S/L v1.0

| NotI-HF® Component List |                              |            |                      |
|-------------------------|------------------------------|------------|----------------------|
| NEB Part Number         | Component Description        | Lot Number | Individual QC Result |
| R3189LVIAL              | NotI-HF®                     | 0041803    | Pass                 |
| B7204SVIAL              | CutSmart® Buffer             | 10013537   | Pass                 |
| B7024SVIAL              | Gel Loading Dye, Purple (6X) | 10013725   | Pass                 |

| Assay Name/Specification   | Lot # 10017520 |
|--|----------------|
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>           After a 10-fold over-digestion of pBC4 DNA with NotI-HF™, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with NotI-HF™.</p>                                   | Pass           |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>           A 50 µl reaction in CutSmart™ Buffer containing 1 µg of pBC4 DNA and a minimum of 200 Units of NotI-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           |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>           A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 200 units of NotI-HF™ incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p> | Pass           |
| <p><b>Endonuclease Activity (Nicking)</b><br/>           A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of NotI-HF™ incubated for 4 hours at 37°C results in &lt;10%</p>   | Pass           |

| Assay Name/Specification  | Lot # 10017520 |
|---|----------------|
| conversion to the nicked form as determined by agarose gel electrophoresis. |                |

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



Tony Spear-Alfonso  
Production Scientist  
10 Jul 2018



Michael Tonello  
Packaging Quality Control Inspector  
09 Aug 2018