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New England Biolabs Certificate of Analysis

| Product Name: | BclI-HF® |
|------------------------|--|
| Catalog #: | R3160S/L |
| Concentration: | 20,000 units/ml |
| Unit Definition: | One unit is defined as the amount of enzyme required to digest 1 μg of Lambda DNA (dam-) in 1 hour at 37°C in a total reaction volume of 50 μl. |
| <i>Lot</i> #: | 0021801 |
| Assay Date: | 01/2018 |
| Expiration Date: | 1/2020 |
| Storage Temp: | -20°C |
| Storage Conditions: | 300 mM NaCl , 10 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 500 μg/ml BSA, (pH 7.4 @ 25°C) |
| Specification Version: | PS-R3160S/L v1.0 |
| Effective Date: | 22 May 2017 |

| Assay Name/Specification (minimum release criteria) | Lot #0021801 |
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| Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in CutSmart® Buffer containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of BcII-HF incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Functional Testing (15 minute Digest) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda dam- DNA and 1 µl of BclI-HF incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis. | Pass |
| Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of Lambda dam- DNA with BclI -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 BclI-HF. | Pass |
| Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda dam- DNA and a minimum of 60 units of BcII-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 |
| Protein Purity Assay (SDS-PAGE) - BclI-HF is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |

* 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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Authorized by Derek Robinson 22 May 2017



Stephanie Onetto

Inspected by Stephanie Cornelio 12 Jan 2018