

## New England Biolabs Certificate of Analysis

**Product Name:** BstEII-HF™  
**Catalog #:** R3162S/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 in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Lot #:** 0011304  
**Assay Date:** 04/2013  
**Expiration Date:** 04/2015  
**Storage Temp:** -20 °C  
**Storage Conditions:** 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R3162S/L v1.0  
**Effective Date:** 17 Apr 2013

| Assay Name/Specification (minimum release criteria)   | Lot #0011304 |
|---|--------------|
| <b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of BstEII-HF™ incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.                  | <b>Pass</b>  |
| <b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 60 units of BstEII-HF™ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | <b>Pass</b>  |
| <b>Ligation and Recutting (Terminal Integrity)</b> - After a 10-fold over-digestion of Lambda DNA with BstEII-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 BstEII-HF™.  | <b>Pass</b>  |
| <b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 60 units of BstEII-HF™ incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.     | <b>Pass</b>  |

\* 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.

M. W. Southworth

*Bo Wu*

Authorized by  
Maurice Southworth  
17 Apr 2013

Inspected by  
Bo Wu  
19 Dec 2013

