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Date

04 Nov 2016

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## New England Biolabs Product Specification

Product Name: BsiWI-HF®

Catalog #: R3553S/L

Concentration: 20,000 units/ml

Unit Definition:

One unit is defined as the amount of enzyme required to digest 1 µg of PhiX174 DNA in 1 hour at 37°C in a total reaction

volume of 50  $\mu$ l.

Shelf Life: 24 months
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-R3553S/L v1.0
Effective Date: 04 Nov 2016

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in CutSmart® Buffer containing 1  $\mu$ g of supercoiled pUC19 DNA and a minimum of 20 units of BsiWI-HF incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - 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 100 units of BsiWI-HF incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of PhiX174 DNA and 1 µl of BsiWI-HF incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of PhiX174 DNA with BsiWI-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 BsiWI-HF.

Non-Specific DNase Activity (16 Hour) - A 50 μl reaction in CutSmart® Buffer containing 1 μg of PhiX174 DNA and a minimum of 100 units of BsiWI-HF incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - BsiWI-HF is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

Derek Robinson

Director of Quality Control







<sup>\*</sup> 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.