

## New England Biolabs Product Specification

Product Name:	BmtI-HF <sup>®</sup>
Catalog #:	R3658S/L
Concentration:	20,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 µg of pXba in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version:	PS-R3658S/L v3.0
Effective Date:	10 Oct 2022

### Assay Name/Specification (minimum release criteria)

**Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in rCutSmart<sup>™</sup> 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 BmtI-HF<sup>®</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (15 minute Digest)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of pXba DNA and 1 µl of BmtI-HF<sup>®</sup> 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 pXba DNA with BmtI-HF<sup>®</sup>, >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 BmtI-HF<sup>®</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart<sup>™</sup> Buffer containing 1 µg of pXba DNA and a minimum of 100 units of BmtI-HF<sup>®</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 20 units of BmtI-HF<sup>®</sup> is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

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Date 10 Oct 2022

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