240 County Road
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# New England Biolabs
# Product Specification

| | |
|---|---|
| *Product Name:* | *BsmFI* |
| *Catalog #:* | *R0572S/L* |
| *Concentration:* | *2,000 units/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 DNA 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, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-R0572S/L v2.0* |
| *Effective Date:* | *12 Jan 2024* |

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of BsmFI 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 rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ $^{3}$H] *E. coli* DNA and a minimum of 20 units of BsmFI 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™ Buffer containing 1 µg of pBR322 DNA and 1 µl of BsmFI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

**Ligation and Recutting (Terminal Integrity)** - After a 5-fold over-digestion of pBR322 DNA with BsmFI, >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 BsmFI.

**Non-Specific DNase Activity (16 hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBR322 DNA and a minimum of 2 units of BsmFI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE: although no nuclease degradation is detected under these conditions, extended incubations and/or high concentrations of this enzyme may result in star activity. See the product FAQ for recommended reaction conditions for this enzyme.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 2 units of BsmFI is screened for the presence of *E. coli* genomic DNA using SYBR® 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 12 Jan 2024

Nancy Considine
Quality Approver

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices