240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Product Specification

Product Name: TspRI
Catalog #: R0582S

Concentration: 10,000 units/ml

Unit Definition:

One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 65°C

in a total reaction volume of 50 µl.

Shelf Life: 24 months
Storage Temp: -20°C

Storage Conditions: 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @)

25°C,

Specification Version: PS-R0582S v2.0 Effective Date: 15 Aug 2023

Assay Name/Specification (minimum release criteria)

Exonuclease Activity (Radioactivity Release) - A 50 μl reaction in rCutSmartTM Buffer containing 1 μg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 50 units of TspRI incubated for 4 hours at 65°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 μl reaction in rCutSmartTM Buffer containing 1 μg of Lambda DNA and 1 μl of TspRI incubated for 15 minutes at 65°C results in complete digestion as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 μl reaction in rCutSmartTM Buffer containing 1 μg of Lambda DNA and a minimum of 50 units of TspRI incubated for 16 hours at 65°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 10 units of TspRI 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.

Ligation and Recutting (Terminal Integrity) - After a 10-fold over-digestion of Lambda DNA with TspRI, >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 TspRI.

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







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Date 15 Aug 2023

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