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## New England Biolabs Product Specification

Product Name:	NspI
Catalog #:	R0602S/L/V
Concentration:	10,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda 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:	50 mM KCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton® X-100, 200 μg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version:	PS-R0602S/L/V v2.0
Effective Date:	09 Nov 2023

## Assay Name/Specification (minimum release criteria)

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

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

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in rCutSmart<sup>TM</sup> Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 10 units of NspI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - A 50  $\mu$ l reaction in rCutSmart<sup>TM</sup> Buffer containing 1  $\mu$ g of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 50 units of NspI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50  $\mu$ l reaction in rCutSmart<sup>TM</sup> Buffer containing 1  $\mu$ g of Lambda DNA and a minimum of 50 units of NspI 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 10 units of NspI 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  $\leq 1$  *E. coli* genome.



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Date 09 Nov 2023