

## New England Biolabs Product Specification

*Product Name:* EnGen<sup>®</sup> Seq1 Cas9  
*Catalog #:* M0668T  
*Concentration:* 20  $\mu$ M  
*Shelf Life:* 24 months  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT; 50% Glycerol (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0668T v1.0  
*Effective Date:* 16 May 2023

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

**Endonuclease Activity (Nicking)** - A 50  $\mu$ l reaction in NEBuffer<sup>™</sup> r3.1 containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Seq1 Cas9 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  $\mu$ l reaction in NEBuffer<sup>™</sup> r3.1 containing 1  $\mu$ g of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Seq1 Cas9 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (Targeted Digestion)** - A 20  $\mu$ l reaction in NEBuffer<sup>™</sup> r3.1 containing 20 nM of 550 bp FAM and ROX-labeled double-stranded target DNA, 200 nM sgRNA, and 200 nM EnGen<sup>®</sup> Seq1 Cas9 incubated for 15 minutes at 37°C results in  $\geq$ 90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50  $\mu$ l reaction in NEBuffer<sup>™</sup> r3.1 containing 1  $\mu$ g of Lambda DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Seq1 Cas9 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**RNase Activity (Extended Digestion)** - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 pmol of EnGen<sup>®</sup> Seq1 Cas9 is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

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Quality Approver

