

## New England Biolabs Product Specification

**Product Name:** *EnGen<sup>®</sup> Sau Cas9*  
**Catalog #:** *M0654T*  
**Concentration:** *20 µM*  
**Shelf Life:** *24 months*  
**Storage Temp:** *-20°C*  
**Storage Conditions:** *20 mM Tris-HCl, 300 mM NaCl, 0.1 mM TCEP, 50% Glycerol, (pH 7.5 @ 25°)*  
**Specification Version:** *PS-M0654T v1.0*  
**Effective Date:** *11 Mar 2019*

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Sau 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 µl reaction in NEBuffer 3.1 containing 1 µg 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> Sau Cas9 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (Targeted Digestion)** - A 20 µl reaction in NEBuffer 3.1 containing 20 nM of 515 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen<sup>®</sup> Sau Cas9 incubated for 15 minutes at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Sau Cas9 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE)** - EnGen<sup>®</sup> Sau Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**RNase Activity (Extended Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 pmol of EnGen<sup>®</sup> Sau 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.



Date 11 Mar 2019

Derek Robinson  
Director of Quality Control

