

New England Biolabs Product Specification

Product Name:	<i>Sulfolobus DNA Polymerase IV</i>
Catalog #:	M0327S/L
Concentration:	2,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 55°C.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	PS-M0327S/L v1.0
Effective Date:	02 Dec 2015

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of *Sulfolobus* DNA Polymerase IV incubated for 4 hours at either 37°C or 55°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 20 units of *Sulfolobus* DNA Polymerase IV incubated for 4 hours at either 37°C or 55°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 2 units of *Sulfolobus* DNA Polymerase IV 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) - *Sulfolobus* DNA Polymerase IV is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 2 units of *Sulfolobus* DNA Polymerase IV 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.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 20 µl reaction in ThermoPol® Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 20 units of *Sulfolobus* DNA Polymerase IV incubated for 30 minutes at either 37°C or 55°C yields <10% degradation as determined by capillary electrophoresis.



New England Biolabs Product Specification



Date 02 Dec 2015

Derek Robinson
Director of Quality Control

