

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>DNA Polymerase I (E. coli)</i>
<b>Catalog #:</b>	M0209S/L/V
<b>Concentration:</b>	10,000 units/ml
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.
<b>Shelf Life:</b>	24 months
<b>Storage Temp:</b>	-20°C
<b>Storage Conditions:</b>	25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
<b>Specification Version:</b>	PS-M0209S/L/V v2.0
<b>Effective Date:</b>	21 Aug 2024

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer 2 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of DNA Polymerase I (*E. coli*) incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Phosphatase Activity (pNPP)** - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units DNA Polymerase I (*E. coli*) incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 10 units of DNA Polymerase I (*E. coli*) 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.

**Protein Purity (Microfluidic Electrophoresis)** - DNA Polymerase I (*E. coli*) is ≥97% pure as determined by microfluidic electrophoresis.

**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 10 units of DNA Polymerase I (*E. coli*) is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

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Date 21 Aug 2024

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Quality Approver

