

## New England Biolabs Certificate of Analysis

*Product Name:* DNA Polymerase I, Large (Klenow) Fragment  
*Catalog #:* M0210M  
*Concentration:* 50,000 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 37°C.  
*Lot #:* 0891603  
*Assay Date:* 03/2016  
*Expiration Date:* 03/2018  
*Storage Temp:* -20°C  
*Storage Conditions:* 25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0210M v1.0  
*Effective Date:* 16 Oct 2015

Assay Name/Specification (minimum release criteria)	Lot #0891603
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in NEBuffer 2 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of DNA Polymerase I, Large (Klenow) Fragment incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -Nitrophenyl Phosphate (pNPP) and a minimum of 100 units DNA Polymerase I, Large (Klenow) Fragment incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - DNA Polymerase I, Large (Klenow) Fragment is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 50 units of DNA Polymerase I, Large (Klenow) Fragment is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>
<b>RNase Activity (Extended Digestion)</b> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of DNA Polymerase I, Large (Klenow) Fragment 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.	<b>Pass</b>



Authorized by  
Melanie Fortier  
16 Oct 2015



Inspected by  
Cathy Rezac  
21 Jun 2016

