

## New England Biolabs Certificate of Analysis

**Product Name:** Klenow Fragment (3'-5' exo-)  
**Catalog Number:** M0212L  
**Concentration:** 5,000 U/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.  
**Packaging Lot Number:** 10069550  
**Expiration Date:** 10/2021  
**Storage Temperature:** -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-M0212S/L v2.0

Klenow Fragment (3'-5' exo-) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0212LVIAL	Klenow Fragment (3'-5' exo-)	10055677	Pass
B7002SVIAL	NEBuffer™ 2	10061303	Pass

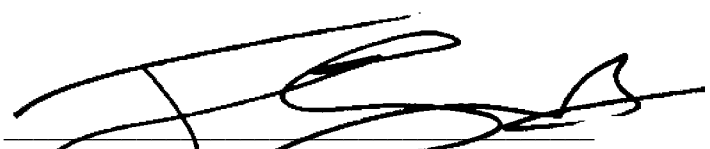
Assay Name/Specification	Lot # 10069550
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b>            A 50 µl reaction in NEBuffer 2 containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 50 units of Klenow Fragment (3'-5' exo-) incubated for 30 minutes at 37°C yields &lt;10% degradation as determined by fluorescent detection.</p>	Pass
<p><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 Klenow Fragment (3'-5' exo-) is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescence detection.</p>	Pass
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            A minimum of 50 units of Klenow Fragment (3'-5' exo-) 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.</p>	Pass

Assay Name/Specification	Lot # 10069550
<p><b>Protein Purity Assay (SDS-PAGE)</b> Klenow Fragment (3'–5' exo-) is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><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 Klenow Fragment (3'–5' exo-) incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 2 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 200 units of Klenow Fragment (3'–5' exo-) incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 50 units of Klenow Fragment (3'–5' exo-) incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p><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 p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Klenow Fragment (3'–5' exo-) incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass

This product has been tested and shown to be in compliance with all specifications.



Christie Vazquez  
Production Scientist  
23 Oct 2019



Josh Hersey  
Packaging Quality Control Inspector  
23 Mar 2020