

## 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:** 10083165  
**Expiration Date:** 05/2022  
**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-)	10073699	Pass
B7002SVIAL	NEBuffer™ 2	10075963	Pass

Assay Name/Specification	Lot # 10083165
<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 # 10083165
<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>	<b>Pass</b>
<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>	<b>Pass</b>
<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>	<b>Pass</b>
<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>	<b>Pass</b>
<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>	<b>Pass</b>

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

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