

## New England Biolabs Certificate of Analysis

**Product Name:** *Klenow Fragment (3'-5' exo-)*  
**Catalog Number:** *M0212M*  
**Concentration:** *50,000 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:** *10255828*  
**Expiration Date:** *02/2026*  
**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-M0212M v2.0*

Klenow Fragment (3'-5' exo-) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0212MVIAl	Klenow Fragment (3'-5' exo-)	10234144	Pass
B7002SVIAl	NEBuffer™ 2	10237091	Pass

Assay Name/Specification	Lot # 10255828
<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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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 <0.1% of the total radioactivity.	<b>Pass</b>
<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.	<b>Pass</b>
<b>Phosphatase Activity (pNPP)</b>	<b>Pass</b>

Assay Name/Specification	Lot # 10255828
<p>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>	
<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>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 fluorescent detection.</p>	<b>Pass</b>
<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>	<b>Pass</b>
<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>	<b>Pass</b>

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

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12 Apr 2024



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