

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: *10146272*
Expiration Date: *03/2024*
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-)	10143543	Pass
B7002SVIAl	NEBuffer™ 2	10143291	Pass

Assay Name/Specification	Lot # 10146272
<p>Non-Specific DNase Activity (16 Hour) 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>Single Stranded DNase Activity (FAM-Labeled Oligo) 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 <10% degradation as determined by fluorescent detection.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) 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 # 10146272
<p>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 1 µl of Klenow Fragment (3'–5' exo-) 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.</p>	Pass
<p>Phosphatase Activity (pNPP) A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ 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 <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) Klenow Fragment (3'–5' exo-) is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>Endonuclease Activity (Nicking) 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.</p>	Pass
<p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 2 containing 1 µg of a mixture of single and double-stranded [³H] 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.</p>	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

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14 Apr 2022

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