

## New England Biolabs Certificate of Analysis

*Product Name:* Klenow Fragment (3'→5' exo-)  
*Catalog #:* M0212M  
*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 #:* 0351803  
*Assay Date:* 03/2018  
*Expiration Date:* 3/2020  
*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-M0212M v1.0  
*Effective Date:* 22 Sep 2015

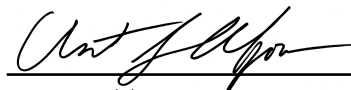
Assay Name/Specification (minimum release criteria)	Lot #0351803
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in NEBuffer 2 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of Klenow Fragment (3'→5' exo-) incubated for 4 hours at 37°C results in <10% conversion to the 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] <i>E. coli</i> 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 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> - 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 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.	<b>Pass</b>
<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.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0351803
<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 50 units of Klenow Fragment (3'→5' exo-) 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 <math>\leq 1</math> <i>E. coli</i> genome.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> - A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 <math>\mu</math>l 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 <math>\mu</math>l 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>



Authorized by  
Melanie Fortier  
22 Sep 2015



Inspected by  
Tony Spear-Alfonso  
09 Mar 2018

