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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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:	10169282
Expiration Date:	08/2024
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0212MVIAL	Klenow Fragment (3'-5' exo-)	10167306	Pass	
B7002SVIAL	NEBuffer™ 2	10162785	Pass	

Assay Name/Specification	Lot # 10169282
<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 $\leq$ 1 E. coli genome.	Pass
Single Stranded DNase Activity (FAM-Labeled Oligo) A 50 $\mu$ I 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.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 $\mu$ l reaction in NEBuffer 2 containing 1 $\mu$ g 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.	Pass





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Assay Name/Specification	Lot # 10169282
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 $\mu$ l reaction in NEBuffer 2 containing 1 $\mu$ g 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.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 $\mu$ I reaction in NEBuffer 2 containing 1 $\mu$ g 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.	Pass
<b>Phosphatase Activity (pNPP)</b> 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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> Klenow Fragment (3' $-5$ ' exo-) is $\geq$ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l 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.	Pass

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

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