NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

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.*
Shelf Life:	*24 months*
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 v2.0*
Effective Date:	*12 Feb 2020*

Assay Name/Specification (minimum release criteria)
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.
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer 2 containing 1 µg of a mixture of single and double-stranded [^{3}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.
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.
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ 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.
Protein Purity Assay (SDS-PAGE) - Klenow Fragment (3'→5' exo-) is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Assay Name/Specification (minimum release criteria)
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.
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.
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.

Date 12 Feb 2020

Derek Robinson
Director, Quality Control