

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Klenow Fragment (3'→5' exo-)</i>
<i>Catalog #:</i>	<i>M0212S/L/V</i>
<i>Concentration:</i>	<i>5,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0212S/L v2.0</i>
<i>Effective Date:</i>	<i>12 Feb 2020</i>

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 [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.

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 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.

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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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 fluorescence 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.

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