New England Biolabs  
Product Specification

**Product Name:** Klenow Fragment (3'→5' exo-)

**Catalog #:** M0212S/L/V

**Concentration:** 5,000 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-M0212S/L 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[^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.
New England Biolabs
Product Specification

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>qPCR DNA Contamination (E. coli Genomic)</strong> - 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.</td>
</tr>
<tr>
<td><strong>RNase Activity (Extended Digestion)</strong> - 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 37ºC. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescence detection.</td>
</tr>
<tr>
<td><strong>Single Stranded DNase Activity (FAM-Labeled Oligo)</strong> - 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 &lt;10% degradation as determined by fluorescent detection.</td>
</tr>
</tbody>
</table>

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

Derek Robinson
Director, Quality Control

Date 12 Feb 2020