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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><br>A minimum of 50 units of Klenow Fragment (3' $-5$ ' exo-) is screened for the presence<br>of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli<br>16S rRNA locus. Results are quantified using a standard curve generated from<br>purified E. coli genomic DNA. The measured level of E. coli genomic DNA<br>contamination is $\leq$ 1 E. coli genome. | Pass           |
| Single Stranded DNase Activity (FAM-Labeled Oligo)<br>A 50 $\mu$ I reaction in NEBuffer 2 containing a 10 nM solution of a fluorescent internal<br>labeled oligonucleotide and a minimum of 50 units of Klenow Fragment (3'-5' exo-)<br>incubated for 30 minutes at 37°C yields <10% degradation as determined by<br>fluorescent detection.  | Pass           |
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 $\mu$ l reaction in NEBuffer 2 containing 1 $\mu$ g of T3 or T7 DNA in addition to a<br>reaction containing Lambda-HindIII DNA and a minimum of 50 units of Klenow Fragment<br>(3'-5' exo-) incubated for 16 hours at 37°C results in a DNA pattern free of<br>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><br>A 50 $\mu$ l reaction in NEBuffer 2 containing 1 $\mu$ g of a mixture of single and<br>double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 200 units of Klenow Fragment<br>(3'-5' exo-) incubated for 4 hours at 37°C releases <0.1% of the total<br>radioactivity.  | Pass           |
| <b>Endonuclease Activity (Nicking)</b><br>A 50 $\mu$ I reaction in NEBuffer 2 containing 1 $\mu$ g of supercoiled PhiX174 DNA and a<br>minimum of 50 units of Klenow Fragment (3' $-5$ ' exo-) incubated for 4 hours at 37°C<br>results in <10% conversion to the nicked form as determined by agarose gel<br>electrophoresis.   | Pass           |
| <b>Phosphatase Activity (pNPP)</b><br>A 200 μl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl2 containing 2.5 mM<br>p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Klenow Fragment (3'–5'<br>exo-) incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase<br>activity as determined by spectrophotometric analysis.                        | Pass           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>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><br>A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA<br>and a minimum of 1 $\mu$ l of Klenow Fragment (3' $-5$ ' exo-) is incubated at 37°C. After<br>incubation for 16 hours, >90% of the substrate RNA remains intact as determined by<br>gel electrophoresis using fluorescent detection. | Pass           |

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

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