NEW ENGLAND BioLabs Inc.

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 #:	*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.*
Lot #:	*0351509*
Assay Date:	*09/2015*
Expiration Date:	*09/2017*
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 v1.0*
Effective Date:	*22 Sep 2015*

Assay Name/Specification (minimum release criteria)	**Lot #0351509**
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.	**Pass**
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.	**Pass**
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 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**
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.	**Pass**
Protein Purity Assay (SDS-PAGE) - Klenow Fragment (3'→5' exo-) is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0351509**
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.	**Pass**
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.	**Pass**
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.	**Pass**

Authorized by
Melanie Fortier
22 Sep 2015

Inspected by
Denisa Gilaj
23 Sep 2015

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices