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## New England Biolabs Certificate of Analysis

Product Name: Vent® (exo-) DNA Polymerase

Catalog Number: M0257L Concentration: 2,000 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 75°C

Packaging Lot Number: 10228311
Expiration Date: 01/2026
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.1 %

Triton®X-100 , 50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-M0257S/L v2.0

Vent® (exo-) DNA Polymerase Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0257LVIAL	Vent® (exo-) DNA Polymerase	10228310	Pass	
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10215580	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	10210679	Pass	

Assay Name/Specification	Lot # 10228311
Endonuclease Activity (Nicking) A 50 μl reaction in ThermoPol® Reaction Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 200 units of Vent® (exo-) DNA Polymerase incubated for 4 hours at 37°C and 75°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 ThermoPol® Reaction Buffer containing 1 μg of a mixture of single and double-stranded [ ³H] E. coli DNA and a minimum of 200 units of Vent® (exo-) DNA Polymerase incubated for 4 hours at 37°C and 75°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 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 2 units of Vent® (exo-) DNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass



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Assay Name/Specification	Lot # 10228311
PCR Amplification (2.0 kb Lambda DNA) A 25 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 0.5 units of Vent® (exo-) DNA Polymerase for 30 cycles of PCR amplification results in the expected 2.0 kb product.	Pass
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 Vent® (exo-) DNA Polymerase 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)  Vent® (exo-) DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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 Vent® (exo-) DNA Polymerase 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 20 µl reaction in ThermoPol® Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 20 units of Vent® (exo-) DNA Polymerase incubated for 30 minutes at 37°C and 75°C yields <10% degradation as determined by capillary electrophoresis.	Pass
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 2 units of Vent® (exo-) DNA Polymerase 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

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

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13 Mar 2024

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19 Mar 2024