

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: 10099433
Expiration Date: 12/2022
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			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0257LVIAL	Vent® (exo-) DNA Polymerase	10092763	Pass
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10086110	Pass
B1003SVIAL	Magnesium Sulfate (MgSO ₄) Solution	10092112	Pass

Assay Name/Specification	Lot # 10099433
Protein Purity Assay (SDS-PAGE) Vent® (exo-) DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) 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
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)	Pass

Assay Name/Specification	Lot # 1009433
<p>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.</p>	
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass

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

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

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12 Feb 2021

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12 Feb 2021