NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*Vent® (exo-) DNA Polymerase*
Catalog Number:	*M0257S*
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:	*10127768*
Expiration Date:	*08/2023*
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
M0257SVIAL	Vent® (exo-) DNA Polymerase	10125081	**Pass**
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10125244	**Pass**
B1003SVIAL	Magnesium Sulfate ($MgSO_4$) Solution	10118450	**Pass**

Assay Name/Specification	Lot # 10127768
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
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**
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**

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Assay Name/Specification	Lot # 10127768
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 200 units of Vent® (exo-) DNA Polymerase incubated for 4 hours at 37°C and 75ºC releases <0.1% of the total radioactivity.	**Pass**
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 75ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**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 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**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**

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

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03 Nov 2021

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