

## 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:** 10123093  
**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 |
| M0257LVIAL                                 | Vent® (exo-) DNA Polymerase                     | 10114986   | Pass                 |
| B9004SVIAL                                 | ThermoPol® Reaction Buffer Pack                 | 10113098   | Pass                 |
| B1003SVIAL                                 | Magnesium Sulfate (MgSO <sub>4</sub> ) Solution | 10118450   | Pass                 |

| Assay Name/Specification   | Lot # 10123093 |
|--|----------------|
| <p><b>Endonuclease Activity (Nicking)</b><br/>           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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   | Pass           |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>           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.</p> | Pass           |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>           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           |

| Assay Name/Specification  | Lot # 10123093 |
|---|----------------|
| <p><b>PCR Amplification (2.0 kb Lambda DNA)</b><br/>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>   | <b>Pass</b>    |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] 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 &lt;0.1% of the total radioactivity.</p>                         | <b>Pass</b>    |
| <p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b><br/>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 &lt;10% degradation as determined by capillary electrophoresis.</p> | <b>Pass</b>    |
| <p><b>RNase Activity (Extended Digestion)</b><br/>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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>             | <b>Pass</b>    |
| <p><b>Phosphatase Activity (pNPP)</b><br/>A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> 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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>           | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Vent® (exo-) DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>  | <b>Pass</b>    |

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](http://www.neb.com/trademarks) for additional information.

*Christie Vazquez*

Christie Vazquez  
Production Scientist  
07 Oct 2021

*Michael Tonello*

Michael Tonello  
Packaging Quality Control Inspector  
07 Oct 2021