

## New England Biolabs Certificate of Analysis

*Product Name:* Vent<sup>®</sup> (exo-) DNA Polymerase  
*Catalog #:* M0257S/L  
*Concentration:* 2,000 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 75°C  
*Lot #:* 0171706  
*Assay Date:* 06/2017  
*Expiration Date:* 6/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 % Triton<sup>®</sup>X-100, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0257S/L v1.0  
*Effective Date:* 17 May 2016

Assay Name/Specification (minimum release criteria)	Lot #0171706
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in ThermoPol <sup>®</sup> Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 200 units of Vent <sup>®</sup> (exo-) DNA Polymerase incubated for 4 hours at either 37°C or 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in ThermoPol <sup>®</sup> Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 200 units of Vent <sup>®</sup> (exo-) DNA Polymerase incubated for 4 hours at either 37°C or 75°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - 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 2 units of Vent <sup>®</sup> (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.	<b>Pass</b>
<b>PCR Amplification (2.0 kb Lambda DNA)</b> - A 25 µl reaction in ThermoPol <sup>®</sup> 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 <sup>®</sup> (exo-) DNA Polymerase for 30 cycles of PCR amplification results in the expected 2.0 kb product.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0171706
<p><b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM <i>p</i>-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Vent<sup>®</sup> (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> - Vent<sup>®</sup> (exo-) DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 2 units of Vent<sup>®</sup> (exo-) DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> - 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<sup>®</sup> (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>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> - A 20 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 20 units of Vent<sup>®</sup> (exo-) DNA Polymerase incubated for 30 minutes at either 37°C or 75°C yields &lt;10% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>



Authorized by  
Melanie Fortier  
17 May 2016



Inspected by  
Tony Spear-Alfonso  
25 May 2017

