

New England Biolabs Certificate of Analysis

Product Name: Vent[®] (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 #: 0171712
Assay Date: 12/2017
Expiration Date: 12/2019
Storage Temp: -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 v1.0
Effective Date: 17 May 2016

Assay Name/Specification (minimum release criteria)	Lot #0171712
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 either 37°C or 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 [³ H] <i>E. coli</i> DNA and a minimum of 200 units of Vent [®] (exo-) DNA Polymerase incubated for 4 hours at either 37°C or 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 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
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

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<p>Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM <i>p</i>-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
<p>Protein Purity Assay (SDS-PAGE) - Vent[®] (exo-) DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 2 units of Vent[®] (exo-) DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR[®] 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>	Pass
<p>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.</p>	Pass
<p>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 either 37°C or 75°C yields <10% degradation as determined by capillary electrophoresis.</p>	Pass



Authorized by
Melanie Fortier
17 May 2016



Inspected by
Tony Spear-Alfonso
01 Dec 2017

