

New England Biolabs Product Specification

Product Name:	<i>Vent</i> [®] DNA Polymerase
Catalog #:	M0254S/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.
Shelf Life:	24 months
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-M0254S/L v1.0
Effective Date:	24 Nov 2015

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking, Polymerase, dNTP) - A 50 µl reaction in ThermoPol[®] Reaction Buffer in the presence of 400 µM dNTPs containing 1 µg of supercoiled pUC19 DNA and a minimum of 20 units of Vent[®] DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

PCR Amplification (2.0 kb Lambda DNA) - A 25 µl reaction in ThermoPol[®] Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 5 ng Lambda DNA with 0.25 units of Vent[®] DNA Polymerase for 25 cycles of PCR amplification results in the expected 2.0 kb product.

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[®] DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - Vent[®] DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 2 units of Vent[®] 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.

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[®] 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.



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Date 24 Nov 2015

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Director of Quality Control

