Deep Vent$_R$® (exo$^-$) DNA Polymerase

#259S	200 units	$75
#259L	1,000 units	$300

Description

Deep Vent$_R$® (exo$^-$) DNA polymerase is the fourth thermostable DNA polymerase available from New England Biolabs.

Deep Vent$_R$® (exo$^-$) DNA polymerase is purified from a strain of *E. coli* containing the Deep Vent® DNA polymerase gene originally isolated from *Pyrococcus sp.* (isolate GB-D). This extremely thermophilic marine archaebacterium was isolated from an ocean submarine thermal vent at 2010 meters where it can grow at temperatures as high as 104°C (1).

Deep Vent$_R$® (exo$^-$) DNA polymerase has been engineered to eliminate the 3'→5' proofreading exonuclease activity associated with Deep Vent$_R$® DNA polymerase (#258).

Advantages of Using Deep Vent$_R$® (exo$^-$) DNA Polymerase

- **Cloned and overexpressed at New England Biolabs**
  Insures high levels of purity and lot-to-lot reproducibility
- **Extremely thermostable**
  Half-life of 23 hours at 95°C; 8 hours at 100°C
  Allows high temperature incubations without loss of enzyme function
  Ideal for primer extensions and thermal cycle DNA sequencing when DNA has a high GC content or complex secondary structure
- **Economical**
  Features a unit definition identical to that of most DNA polymerases
  Less costly on a unit basis than almost all other thermophilic DNA polymerases
- **High Yields**
  Product yield proportionate to amount of enzyme used in the reaction
- **Support reagents and technical information**
  10X reaction buffer, 100 mM $MgSO_4$ and primer extension guidelines accompany every vial of enzyme
  Additional buffer packs available: #007-Vent; 4 vials of 10X buffer, 1 vial of 100 mM $MgSO_4$ and 1 vial of 10 mg/ml non-acetylated BSA for $8.00; 100 mM dNTP reagents also available (page 63 of the 1993/94 NEB catalog)

Non-acetylated BSA is available free of charge when placing an order. The presence of BSA is not necessary, but it has been shown to increase product yields in some cases (e.g. when heme groups are present as in blood samples)

Applications

- **Primer Extension**
  Extends primers up to 15 kb
- **Thermal Cycle DNA Sequencing**
  Excellent alternative to Vent$_R$® (exo$^-$) DNA polymerase for thermal cycle DNA sequencing nanogram quantities of DNA
  Can be used with labeled dATP incorporation and 5' end-label primer protocols as described in the CircumVent™ Thermal Cycle DNA Sequencing Kit. Adjustment of sequencing mix dNTP/ddNTP ratios is suggested for optimal performance (see note). Triton X-100 should be included in both Vent$_R$® (exo$^-$) and Deep Vent$_R$® (exo$^-$) DNA polymerase thermal cycle sequencing reactions as per the instructions included with the CircumVent™ Kit
  Compatible with radiolabel and chemiluminescent detection methods as well as automated sequencers using fluorescein-labeled primers

1X Reaction Buffer Composition (supplied as 10X stock)

10 mM KCl
10 mM $(NH_4)_2SO_4$
20 mM Tris-HCl (pH 8.8 at 24°C)
2 mM $MgSO_4$
0.1% Triton X-100

Selling Concentration

2,000 units/ml

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTP into acid-insoluble material at 75°C in 30 minutes in 1X buffer supplemented with 200 µM each dNTP, ^{3}H-TTP and 0.2 mg/ml activated DNA.

Buffer Notes

The 10X Reaction Buffer supplied with the Vent$_R$® (exo$^-$) DNA polymerase gives a final 1X $MgSO_4$ concentration of 2 mM. This concentration may require adjustment for primer extensions beyond 2 kilobases. For DNA sequencing reactions, a final concentration of 5 mM $MgSO_4$ is recommended, particularly when DNA template material may contain substantial amounts of EDTA. The 1X CircumVent™ Buffer contains 5 mM $MgSO_4$.

DNA Fragment End Conformation

We have tested the effect of Vent$_R$® (exo$^-$) DNA polymerase on three different types of restriction endonuclease cleavages: 5′ overhang (*Eco*R I), 3′ overhang (*Pst* I), and blunt-ended (*Hinc* II). The cleaved DNA was incubated at 72°C for 10 minutes with 2 units of Vent$_R$® (exo$^-$) DNA polymerase in a 13 ml reaction volume (buffer composition: 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8 at 25°) 5 mM $MgSO_4$). The reaction products were analyzed on a 6% polyacrylamide sequencing gel. The 5′ overhang (*Eco*R I

Deep Vent$_R$™ DNA Polymerase

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Description

Deep Vent$_R$™ DNA polymerase is the third thermostable DNA polymerase available from New England Biolabs. The enzyme possesses a strong 3′→5′ proofreading exonuclease, has superior thermostablity, can extend primers for long distances (similar to Vent$_R$™ DNA polymerase and Vent$_R$™ (exo⁻) DNA polymerase), and has now been cloned and overexpressed.

Deep Vent$_R$™ DNA polymerase is purified from a strain of *E. coli* containing the Deep Vent™ DNA polymerase gene originally isolated from *Pyrococcus sp.* (isolate GB-D). This extremely thermophilic marine archaebacterium was isolated from an ocean submarine thermal vent at 2010 meters where it can grow at temperatures as high as 104°C.

Advantages of Using Deep Vent$_R$™ DNA Polymerase

- **Cloned and overexpressed at New England Biolabs**
  Insures high levels of purity and lot-to-lot reproducibility
- **Extremely thermostable**
  Half-life of 23 hours at 95°C; 8 hours at 100°C
  Allows high temperature incubations without loss of enzyme function
  Ideal for primer extensions where DNA has a high GC content or contains hairpin structures
- **Possesses a strong 3′→5′ proofreading exonuclease**
  Proofreading exonuclease activity is 4-fold higher than that of Vent$_R$™ DNA Polymerase (an enzyme proven to have fidelity values 5-15 fold greater than Taq DNA Polymerase). The exact level of DNA polymerization fidelity is currently being assessed
- **Yields long primer extensions, with the longest product length to date of 8.2 Kb**
- **Economical**
  Features a unit definition identical to that of most DNA polymerases
  Less costly on a unit basis than almost all other thermophilic DNA polymerases
- **Very low Km for DNA**
  High affinity between primer:template DNA and Deep Vent$_R$™ DNA polymerase
- **Support reagents and technical information**
  10X reaction buffer, 100 mM $MgSO_4$ and primer extension guidelines accompany every vial of enzyme
  Additional buffer packs available: #007-258; 4 vials of 10X buffer, 1 vial of 100 mM $MgSO_4$ and 1 vial of 10 mg/ml BSA for $8.00; 100 mM dNTP reagents also available (refer to 1992 New England Biolabs catalog)
  Non-acetylated BSA is available free of charge when placing an order. The presence of BSA is not necessary, but it has been shown to increase product yields in some cases (e.g. when heme groups are present as in blood samples)

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DNA Fragment Ends

The ends generated by Deep Vent$_R$™ DNA polymerase are >95% blunt-ended, allowing direct cloning of products. The composition of the ends was determined by extending a 5′-^{32}P-labeled primer on a linear template with Vent$_R$™ DNA polymerase, and analyzing the labeled product on a polyacrylamide sequencing gel. (Note: This is in contrast to the ends generated by Vent$_R$™ (exo-) DNA polymerase where two-thirds of the products are blunt-ended, and the bulk of the remaining products are single base 3′ extensions.)

Technical Data Sheet
7/92

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