Vent$_R$® (exo-) DNA Polymerase

1-800-632-7799
info@neb.com
www.neb.com

M0257S 017120614061

M0257S

200 units **2,000 U/ml** **Lot: 0171206**

RECOMBINANT Store at –20°C Exp: 6/14

Description: Vent$_R$ (exo$^-$) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Vent$_R$ DNA Polymerase (3). This is the preferred form for high-temperature dideoxy sequencing reactions and for high yield primer extension reactions. The fidelity of polymerization by this form is reduced to a level about 2-fold higher than that of *Taq* DNA Polymerase (1,2).

Source: An *E. coli* strain that carries the Vent (D141A / E143A) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus litoralis* (4). The native organism is capable of growth at up to 98°C and was isolated from a submarine thermal vent (5).

Applications:
- PCR
- Primer extension
- Thermal cycle sequencing
- High temperature dideoxy-sequencing

Supplied in: 100 mM KCl, 0.1 mM EDTA, 10 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 0.1% Triton® X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X ThermoPol™ Reaction Buffer
100 mM $MgSO_4$.

Reaction Conditions: 1X ThermoPol Reaction Buffer, with or without additional $MgSO_4$, DNA template, dNTPs, primer and 2–4 units polymerase in a final volume of 100 µl.

1X ThermoPol Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
2 mM $MgSO_4$
0.1% Triton X-100
pH 8.8 @ 25°C

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.

Unit Assay Conditions: 1X ThermoPol Reaction Buffer, 200 µM each dNTP including [^{3}H]-dTTP, 200 µg/ml activated calf thymus DNA.

Heat Inactivation: No

Quality Control Assays

Exonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 200 units of Vent$_R$ (exo-) DNA Polymerase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 75°C releases < 0.1% of total radioactivity

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Vent$_R$ (exo-) DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´→ 5´ degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 200 units of Vent$_R$ (exo-) DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Calculated Half-lives at 95°C:

Deep Vent$_R$™ DNA Polymerase	23 hours
Vent$_R$ DNA Polymerase	6.7 hours
Taq DNA Polymerase	1.6 hours

(See other side)

CERTIFICATE OF ANALYSIS

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References:

1. Mattila, P. et al. (1991) *NAR*, 19:4967–4973.
2. Eckert, K.A. and Kunkel, T.A. et al. (1991) *PCR Methods and Applications* 1, 17–24.
3. Kong, H.M. et al. (1993) *J. Biol. Chem.* 268, 1965–1975.
4. Perler, F.B. et al. (1992) *PNAS USA* 89:5577–5581.
5. Belkin, S. and Jannasch, H.W. (1985) *Arch Microbiol.* 141, 181–186.

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

Diluent D
#B8004S 4.0 ml

BSA
#B9001S 6.0 ml

ThermoPol Reaction Buffer Pack
#B9004S 6.0 ml

ThermoPol II (Mg-free) Reaction Buffer Pack
#B9005S 6.0 ml

ThermoPol DF (Detergent-free) Reaction Buffer Pack
#B9013S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

Using NEB Thermophilic DNA Polymerases to Extend a Primer

General Approach–Setting up a Primer Extension Reaction or a PCR Reaction: Basic reaction conditions are 1X ThermoPol Reaction Buffer, DNA template, DNA polymerase, 1–6 mM $MgSO_4$ (see suggested initial conditions), 200–400 µM each dNTP and 0.4 µM primer.

The three most important variables to optimize are the amount of polymerase, the annealing temperature for the primer and the magnesium level. Each new primer: template may require reoptimization.

Enzyme Amount: It is important to use the optimal amount of enzyme, especially with the proofreading DNA polymerases. Start with 1 unit/100 µl reaction volume for proofreading DNA polymerases or 4 units/100 µl reaction volume for exo$^-$ derivatives (for different reaction volumes adjust this ratio accordingly). In general, lower DNA template concentrations in a primer extension reaction necessitate using the lower amount of DNA polymerase within the recommended range.

Recommended ranges are 1–2 units per 100 µl reaction volume for the Vent$_R$, and Deep Vent$_R$ DNA polymerases, and 2–4 units for the Vent$_R$ (exo$^-$) and Deep Vent$_R$ (exo$^-$) DNA Polymerases.

Annealing Temperature: The optimal annealing temperature for the primer can usually be predicted from any of several standard methods of calculation. If this temperature does not give satisfactory results, the annealing temperature should be examined in 3°C increments.

In general, the Vent$_R$ and Deep Vent$_R$ DNA polymerases use annealing temperatures that tend to be the same, or higher, than annealing temperatures used by other DNA polymerases. (Different annealing temperatures may be required by different polymerases, perhaps due to differences in the K_m for binding DNA).

Magnesium Concentration: The optimal magnesium concentration is usually 2, 4 or 6 mM. If EDTA is present at significant levels in DNA added to your reaction, the test range may need to be extended higher. For Vent$_R$ and Deep Vent$_R$ DNA Polymerases, primer extensions longer than 2 kb almost always require magnesium levels higher than 2 mM, while for primer extensions shorter than 2 kb, there is no correlation between length and optimum magnesium concentration.

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ThermoPol II (Mg-free) Reaction Buffer Pack
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ThermoPol DF (Detergent-free) Reaction Buffer Pack
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