M0257S
200 units 2,000 U/ml Lot: 0171212
RECOMBINANT Store at –20°C Exp: 12/14

Description: VentR (exo–) DNA Polymerase has been genetically engineered to eliminate the 3´→5´ proofreading exonuclease activity associated with Vent, 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 deoxysequencing

Reagents Supplied with Enzyme: 10X ThermoPol Reaction Buffer 100 mM MgSO4,

Reaction Conditions: 1X ThermoPol Reaction Buffer, with or without additional MgSO4, DNA template, dNTPs, primer and 2–4 units polymerase in a final volume of 100 µl.

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.

Heat Inactivation: No

Quality Control Assays
Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 200 units of VentR (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: 23 hours
- VentR DNA Polymerase: 6.7 hours
- Taq DNA Polymerase: 1.6 hours

(See other side)
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 MgSO₄, (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 exonuclease 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, and Deep Vent DNA polymerases, and 2–4 units for the Vent, (exo⁻) and Deep Vent, (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, and Deep Vent 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 Kₘ 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, and Deep Vent 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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