One unit is defined as the amount of 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

**Unit Assay Conditions:**
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.

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**Quality Control Assays**

**Exonuclease Activity:**
Incubation of a 50 µl reaction in ThermPol Reaction Buffer containing a minimum of 200 units of Vent (exo-) DNA Polymerase with 1 µg of supercoiled φX174 DNA for 4 hours at 75°C results in > 95% 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™ DNA Polymerase: 23 hours
- Vent™ DNA Polymerase: 6.7 hours
- Taq DNA Polymerase: 1.6 hours

(See other side)
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 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 Vent⁰, and Deep Vent⁰, 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. We recommend using NEB’s Tm Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

In general, the Vent⁰ and Deep Vent⁰ 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).

References:

Companion Products Sold Separately:
Magnesium Sulfate (MgSO₄) Solution
#B1003S 6.0 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

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