



Vent_R[®] DNA Polymerase

#254S 200 units\$75
#254L 1,000 units\$300

Description

Vent[®] DNA polymerase constitutes the first member of the New England Biolabs family of highly thermophilic DNA polymerases. While Vent[®] DNA polymerase was originally purified from the extreme thermophile *Thermococcus litoralis*, an archaeobacterium growing at temperatures up to 98°C in thermal vents on the ocean floor (1), the enzyme is now purified from a strain of *E. coli* containing the Vent[®] DNA polymerase gene (2).

The Vent DNA polymerase family consists of Vent[®] DNA polymerase (#254), Vent[®] (exo⁻) DNA polymerase (#257), Deep Vent[®] DNA polymerase (#258) and Deep Vent[®] (exo⁻) DNA polymerase (#259). All three DNA polymerases are alike in being very thermostable and capable of extending primers up to 8-15 kb in length. Vent_R[®] DNA polymerase should be used when a high level of fidelity is desired. It possesses a documented level of fidelity that is 5-15 fold higher than that of Taq DNA polymerase (3-6). Further positive features of interest are listed below.

Amongst the other members of the Vent[®] DNA polymerase family, the Vent_R[®] (exo⁻) DNA polymerase is the enzyme of choice for high temperature dideoxy sequencing, thermal cycle sequencing (CircumVent™ sequencing kit is available), and primer extensions where a high product yield is desired and a fidelity level 2-fold better than Taq DNA Polymerase is acceptable. The Deep Vent_R[®] DNA polymerase is the enzyme of choice for primer extensions requiring extremely high temperature exposures and a high level of fidelity. Separate technical bulletins are available for these DNA polymerases.

Note: Vent_R[®] DNA polymerases cannot incorporate across from inosine or deoxyuridine in the template strand.

Advantages of Using Vent_R[®] DNA Polymerase

- **Cloned and overexpressed at New England Biolabs**
Insures high levels of purity and lot-to-lot reproducibility
- **Highly thermostable**
Half-life of 6.7 hours at 95°C; 1.8 hours at 100°C
Allows high temperature incubations without loss of enzyme function
Ideal for primer extensions where DNA has a high GC content, contains hairpin structures, or DNA is methylated
- **Possesses a strong 3'→5' proofreading exonuclease responsible for a documented high level of fidelity**

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- **Yields very long primer extensions, with the longest product length to date of 13.2 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, as low as 30¢/unit based on large pack price
- **Produces blunt-ended DNA fragments (7,8,9), allowing direct cloning of extension products**
- **Active over a wide range of temperatures**
Primer extension usually done at 72°-75°C; however, the polymerase can be used at different temperatures to increase read-through in areas of secondary structure or manipulate strand displacement properties on circular templates
Relative activities at various temperatures: at 40°C activity is 15% of that seen at 75°C; at 50°C, 30%; at 60°C, 50%; and at 80°C, 125%
Primer extensions possible up to 86°C with some primer:template systems
- **Extension temperature determines presence or absence of strand displacement properties on circular templates**
At 72°C, will displace more than 100 bases from the 5' end of an encountered DNA strand or the 5' end of its own primer when a single-stranded circular DNA template is used. (Note: strand-displacement does not result in degradation of the DNA, thus its activity differs from the 5'→3' exonuclease activity of Taq DNA polymerase)
At 55°C, will *not* displace DNA and polymerization is stopped at the encountered 5' end of DNA. The slower rate of primer extension at this temperature (40% of activity normally seen at 75°C) can be compensated for by multiplying normal extension time by 2.5. Also, note that Vent[®] (exo⁻) DNA polymerase shows slightly different characteristics
- **Support reagents and technical information**
10X reaction buffer, 100 mM MgSO₄ 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 MgSO₄ and 1 vial of 10 mg/ml non-acetylated BSA for \$8.00). 100 mM dNTP reagents also available (refer to page 63 of the 1993/94 NEB catalog)

- **Support reagents and technical information (continued)**
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)

Further characterization of Vent₂⁺ DNA polymerase is detailed in reference 10.

Fidelity

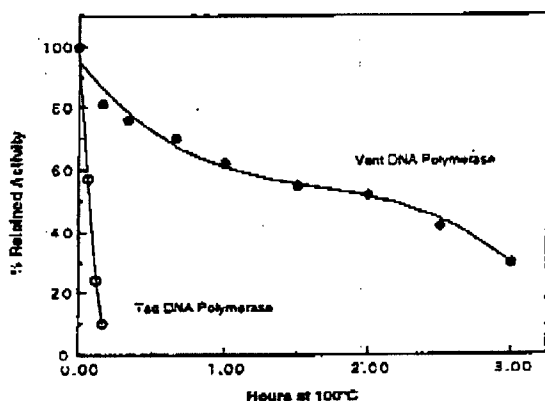
The Vent₂⁺ DNA polymerase has a 3'→5' exonuclease activity that is responsible for the high fidelity of Vent₂⁺ DNA polymerase. Many comparative polymerase fidelity studies by a variety of approaches have been completed (3-6). The fidelity for Vent₂⁺ DNA polymerase is 5-15 fold greater than that of Taq DNA polymerase, while the fidelity for Vent₂⁺ (exo⁻) DNA polymerase is 2-fold greater than that of Taq DNA polymerase.

DNA Fragment Ends

The ends generated by Vent₂⁺ DNA polymerase are >95% blunt-ended, allowing direct cloning of products. The composition of the ends was determined by extending a 5'-³²P-labeled primer on a linear template with Vent₂⁺ DNA polymerase, and analyzing the labeled product on a polyacrylamide sequencing gel. (Note: This is in contrast to the ends generated by Vent₂⁺ (exo⁻) DNA polymerase where two-thirds of the products are blunt-ended, and the bulk of the remaining products are single base 3' extensions.)

Heat Stability

Vent₂⁺ DNA Polymerase can be boiled for several hours and still retain much of its activity. The graph below illustrates the differing levels of thermal stability between Taq and Vent₂⁺ DNA polymerases.



DNA Polymerase Thermal Stability: Taq and Vent⁺ DNA polymerases were incubated at 100°C in either Taq or Vent⁺ DNA polymerase buffer, BSA, DNA and dNTP's. Samples were removed from the incubations at the indicated times and assayed for polymerase activity in the presence of fresh assay reagents with ³H-TTP present.

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

10 mM KCl
10 mM (NH₄)₂SO₄
20 mM Tris-HCl (pH 8.8 at 24°C)
2 mM MgSO₄
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, ³H-TTP and 0.2 mg/ml activated DNA.

References

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6. Mattila, P., et al. (1991) *Nucleic Acids Research*, 19:18, 4967-4973.
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10. Kong, H., Kucera, R.B. and Jack, W.E. (1993) *Journal of Biological Chemistry*, 268:3, 1965-1975.

Vent_R[®] DNA Polymerases: Frequently Asked Questions

What Vent_R[®] support reagents are available from New England Biolabs?

Supplied with every Vent_R DNA polymerase vial are the following:

- 10X Vent Reaction Buffer
- 100 mM MgSO₄
- An applications sheet and data card

Non-acetylated BSA is available separately (free of charge, when placing an order) if you wish to perform primer extension reactions with BSA present. Do not use acetylated BSA with reactions above 85°C.

Also available separately:

- Vent Buffer Packs, including:
 - 4 vials of 10X Reaction Buffer,
 - 1 vial of MgSO₄ and
 - 1 vial of non-acetylated BSA (#007-Vent)

- 1X Vent Diluent/Storage Buffer (#008-Vent)

- 100 mM solutions of dNTPs (page 63 of the 1993/94 NEB Catalog)

What can I expect if I switch from another DNA polymerase to one of the Vent_R DNA polymerases?

Product yields should be similar to those seen with other thermophilic DNA polymerases and may be higher (especially if using Vent_R (exo⁻) DNA polymerase). You will need to use the 10X buffer accompanying the enzyme; while Taq DNA polymerase often works well in Vent_R buffer, Vent_R DNA polymerase does not work well in commercially available Taq buffers.

Positive results are usually achieved immediately by following the suggested guidelines for primer extension. Some template:primer systems may require optimization of annealing temperatures or MgSO₄ levels.

How can I get primer extension products in the 4-15 kb range with Vent_R DNA polymerases?

1. Use the right amount of enzyme. For the proofreading Vent_R DNA polymerases, less is sometimes better. For Vent_R (exo⁻) however, use 4-16 units per 100 ul reaction volume (for smaller reaction volumes, scale down the amount of enzyme appropriately).
2. Use the correct primer extension time calculated by the "one minute per kilobase extension product" guideline. When using the proofreading Vent_R DNA polymerases, do not exceed the calculated extension time by more than 20%.
3. Test at least 3 different MgSO₄ levels over the range of 2 mM to 6 mM. Primer extension products above 2 kb often require a higher level of magnesium than the basal level of 2 mM present in the 1X reaction buffer.
4. Use stringent (high) annealing temperatures so that nonspecific priming events do not occur.

What if my primer extension reaction with Vent_R DNA polymerase yields no product?

1. Follow the primer extension guidelines for reaction buffer, amount of enzyme and extension time.
2. Try different annealing temperatures. It is not uncommon for Vent_R DNA polymerase to require a higher annealing temperature than Taq DNA polymerase to achieve the same level of specificity—perhaps a reflection of the differences in their Km for DNA or in their buffer compositions. A higher annealing temperature should always be tried even if the gel lane appears blank by ethidium bromide staining, since nonspecific priming events are not always easily visualized.
3. Purify the DNA by phenol/chloroform extraction and alcohol precipitation. Avoid high salt carry-over into your Vent_R DNA polymerase primer extension reaction.
4. Check your dNTP solution to see if it has undergone hydrolysis.
5. If using BSA, make sure it is non-acetylated BSA and not the acetylated BSA that accompanies our restriction endonucleases.
6. Occasionally a template:primer system is sensitive to BSA. Try your primer extension in the absence of BSA.

What if my primer extension reaction with Vent_R DNA polymerase yields a smear instead of a specific product band?

1. Review the primer extension guidelines. A smear centered at an expected fragment length by gel analysis indicates insufficient magnesium; test higher levels of MgSO₄.
2. A smear directly below an expected fragment length indicates possible dNTP depletion which causes deregulation of the proofreading exonuclease moiety of Vent_R or Deep Vent_R DNA polymerase. Try stopping the experiment sooner or using less template DNA.
3. A smear extending down from the gel well usually indicates some reagent or condition in excess. Use less enzyme (especially important if using the proofreading Vent_R DNA polymerases), primer and template.
4. Don't overdo the primer extension time (stick to the 1 minute/kb rule).

What causes an occasional smear in a "negative control" with no template present?

DNA polymerases with low Km values for DNA, especially those with proofreading exonuclease functions, can cause primer artifacts to form if the DNA polymerase cannot bind to its preferred substrate (a 3' end of an annealed primer). This primer artifact DNA possesses single-stranded and double-stranded regions, and can appear either as DNA barely migrating out of the gel well or as a smear originating at the gel well. Decreasing primer concentration or the length of the experiment, or using Vent_R (exo⁻) DNA polymerase can minimize this artifact.