TECHNICAL DATA SHEET

VerityPfu™ DNA Polymerase

Catalog Numbers: 31-5020-0200U
31-5020-1000U

PRODUCT INFORMATION

Contents: 31-5020-0200U (200 units)
VerityPfu™ DNA Polymerase (2 U/µL): 1 x 100 µL
VerityPfu™ DNA Polymerase Reaction Buffer (5X): 3 x 1.0 mL

31-5020-1000U (1000 units)
VerityPfu™ DNA Polymerase (2 U/µL): 5 x 100 µL
VerityPfu™ DNA Polymerase Reaction Buffer (5X): 15 x 1.0 mL

Use By: 6 months from date of receipt

DESCRIPTION

VerityPfu™ DNA Polymerase is a proprietary next generation, high fidelity, proofreading enzyme. It replicates DNA at 75°C, catalyzing template dependent polymerization of free nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium ions. The VerityPfu DNA polymerase was developed using site directed mutagenisis that allowed for the positive selection of a very high fidelity *Pfu* variant optimized for use at room temperature. Accordingly, the VerityPfu enzyme possesses exceptional 3´→5´ exonuclease, or proofreading, activity that results in 50X higher fidelity than standard *Taq* DNA polymerase. It does not require hot start conditions, allowing easy room temperature PCR reaction set up. Consequently VerityPfu polymerase, when used together with its proprietary buffer system, is highly recommended for use in reactions that require high fidelity synthesis. For added convenience and value, the advanced Reaction Buffer (5X) includes dNTPs and $MgCl_2$.

Due to genetic mutations conferring exceptional proofreading capabilities, VerityPfu is especially effective when very high levels of fidelity are required. This polymerase reliably generates amplicons up to 10 kb under both fast and standard PCR cycling conditions. The resultant amplicons are blunt-end finished and can be used in a variety of downstream applications. Use VerityPfu DNA Polymerase to accurately and reliably amplify DNA from complex genomic, viral, and plasmid templates, and for site directed mutagenesis, long range PCR and next generation sequencing applications, with little to no protocol modification.

STORAGE

Store kit at -20°C upon arrival and limit exposure to light. The kit may undergo up to 30 freeze/thaw cycles without loss of activity. When stored correctly this kit will retain activity for up to 6 months. This product can be stored at 4°C for up to 1 month.

BIOLOGICAL SOURCE

Tonbo's VerityPfu DNA polymerase enzyme is a single recombinant polypeptide of bacterial origin having a molecular weight of ~90 kDa, originally derived from the hyperthermophilic archaeum *Pyrococcus furiosus*.

APPLICATION NOTES

Reaction Buffer: VerityPfu DNA Polymerase Reaction Buffer (5X) contains 15 mM $MgCl_2$, 5 mM dNTPs, plus a proprietary mix of stabilizers and enhancers. This Reaction Buffer has been rigorously developed for optimal PCR success rate, yield and efficiency. We do not recommend introducing additional $MgCl_2$ or enhancers to the reaction mix.

Primers: We recommend that primers have a predicted melting temperature of approximately 60°C using default Primer 3 settings (http://bioinfo.ut.ee/primer3/). For each reaction, a final primer concentration of 0.2 - 0.6 µM is suggested.

Template: For cDNA templates, use less than 100 ng per reaction. For eukaryotic DNA templates, use 5 - 500 ng per reaction.

Annealing Temperature: It is preferable to generate a temperature gradient in order to empirically determine the optimal annealing temperature for the reaction. Otherwise, one can start with an annealing temperature of 55°C and, if non-specific products are observed, increase in 2°C increments (up to maximum 65°C) until an optimal temperature is reached.

Extension Temperature: We observe optimal extension at 72°C. Extension time depends on both the template complexity and amplicon length. For amplicons from eukaryotic genomic DNA or cDNA, we recommend 30 seconds per kb.

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REACTION SETUP / QUICK PROTOCOL

1. Ensure all components are thawed and mixed well. Equilibrate VerityPfu Reaction Buffer to room temperature. Refer to Table 1 for reaction preparation. If preparing multiple reactions, assemble all common components into a master reaction mix. If working with final reaction volumes less than 50 µL, scale component volumes accordingly.
2. As applicable, transfer the recommended volume of master reaction mix, primers and sample template DNA to individual PCR tubes or plates, seal and spin briefly to mix.
3. Refer to the cycling conditions (Table 2) to perform the PCR.

Table 1. Reaction Preparation

Reagent	50 µL reaction	Final Concentration	Notes
5x Reaction Buffer	10.0 µL	1x	
Forward Primer (10 µM)	2.0 µL	400 nM	See above for optimal primer design
Reverse Primer (10 µM)	2.0 µL	400 nM	
Template DNA	<100 ng cDNA <500 ng genomic	variable	See above for template considerations
VerityPfu™ DNA Polymerase (2U/µL)	0.5 µL		
Nuclease free dH_2O	Up to 50 µL final volume		

Table 2. Cycling Conditions

Cycles	Temperature	Time	Notes
1	95°C	1 minute	Initial denaturation
25-35	95°C	15 seconds	Denaturation
	55°C - 65°C	15 seconds	Anneal
	72°C	30 seconds per kb	Extension (30 seconds per kb)

TECHNICAL SUPPORT

Please provide the following information to support@tonbobio.com for troubleshooting and technical support:

- Catalog and batch numbers
- Reaction set-up (master mix)
- Cycling conditions
- Amplicon size
- Screen shots of gel images
- Detailed description of the issue

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