TECHNICAL DATA SHEET

Classic++™ *Taq* DNA Polymerase

Catalog Numbers: 31-5000-0500U
31-5000-2000U

PRODUCT INFORMATION

Contents: 31-5000-0500U (500 units)
Classic++™ *Taq* DNA Polymerase (5 U/µL): 1 x 100 µL
Classic++™ *Taq* Reaction Buffer (5X): 4 x 1.0 mL

31-5000-2000U (2000 units)
Classic++™ *Taq* DNA Polymerase (5 U/µL): 4 x 100 µL
Classic++™ *Taq* Reaction Buffer (5X): 16 x 1.0 mL

Use By: 6 months from date of receipt

DESCRIPTION

Classic++™ *Taq* DNA Polymerase is a next generation thermostable *Taq* polymerase of recombinant origin that possesses 5'→3' polymerase activity, but not 3′→5′ proofreading, exonuclease activity. Convenient and reliable, Classic++ *Taq* polymerase is ideal for standard PCR protocols and has been engineered to provide enhanced speed, yield and specificity over that of standard *Taq* DNA polymerase. In the presence of Tonbo's optimized Classic++ *Taq* Reaction Buffer and your primer(s), this polymerase will synthesize double-stranded DNA from a wide variety of single stranded templates under standard or fast PCR conditions. For added convenience and value, the advanced Reaction Buffer (5X) includes dNTPs and $MgCl_2$.

Classic++ *Taq* polymerase has a non-template-dependent terminal transferase activity that adds a 3' A overhang to the fragment, useful for downstream TA cloning. This polymerase may be used in a wide variety of applications, including high-throughput and crude sample PCR, with little to no protocol modification. Use Classic++ *Taq* DNA Polymerase for the amplification of DNA from GC and AT rich regions from complex genomic, viral, and plasmid templates, as well as in RT-PCR.

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 Classic++ *Taq* DNA polymerase enzyme is a single recombinant polypeptide of bacterial origin having a molecular weight of ~94 kDa, originally derived from the YT-1 strain of *Thermus acquaticus*.

APPLICATION NOTES

Reaction Buffer: Classic++ *Taq* DNA Polymerase Reaction Buffer (5X) contains 15 mM $MgCl_2$, 5 mM dNTPs plus a proprietary mix of stabilizers and enhancers. Tonbo's Classic++ *Taq* 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.

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Extension Temperature: We observe optimal extension at 72°C. Extension time depends on both the template complexity and amplicon length. For amplicons between 1 - 6 kilobases (kb) from eukaryotic DNA, we recommend 15 seconds per kb, and a 1 second extension for amplicons shorter than 1 kb.

REACTION SETUP / QUICK PROTOCOL

1. Ensure all components are thawed and mixed well.
2. Refer to Table 1 for reaction preparation. If preparing multiple reactions, assemble all common components into a master mix. If working with final reaction volumes less than 50 µL, scale component volumes accordingly.
3. As applicable, transfer the recommended volume of master mix, primers and sample template DNA to individual PCR tubes or plates, seal and spin briefly to mix. 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
Taq DNA Polymerase (5U/µL)	0.25 µL - 1.0 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
40	95°C	15 seconds	Denaturation
	55°C - 65°C	15 seconds	Anneal
	72°C	1 to 90 seconds	Extension (15 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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