TECHNICAL DATA SHEET

Classic++™ *Taq* DNA Polymerase Master Mix

Catalog Numbers: 31-5001-0200R
31-5001-1000R

PRODUCT INFORMATION

Contents: 31-5001-0200R (200 rxns)
Classic++™ *Taq* DNA Polymerase Master Mix (2X): 5 x 1.0 mL

31-5001-1000R (1000 rxns)
Classic++™ *Taq* DNA Polymerase Master Mix (2X): 25 x 1.0 mL

Use By: 6 months from date of receipt

DESCRIPTION

Tonbo's Classic++™ *Taq* DNA Polymerase Master Mix is a premixed, ready-to-use cocktail optimized for efficient amplification of DNA templates by PCR. Classic++ *Taq* DNA Polymerase is a next generation thermostable *Taq* DNA 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 Master Mix and your primer(s), the Classic++ *Taq* polymerase will synthesize double-stranded DNA from a wide variety of single stranded templates under standard or fast PCR conditions.

Classic++ *Taq* DNA polymerase has a non-template-dependent terminal transferase activity that adds a 3' A overhang to the fragment, useful for downstream TA cloning. The enzyme is provided in a robust, convenient high performance master mix optimized to enhance standard PCR speed, yield and specificity. It may be used in a wide variety of applications, including high-throughput and crude sample PCR, with little to no protocol modification. Use Tonbo's Classic++ *Taq* DNA Polymerase Master Mix 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. This product may undergo up to 30 freeze/thaw cycles without loss of activity. When stored correctly this product will retain activity for up to 6 months. The 2X Master Mix 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

Master Mix: Classic++ *Taq* DNA Polymerase Master Mix (2X) contains Classic++ *Taq* DNA Polymerase, 6 mM $MgCl_2$, 2 mM dNTPs plus a proprietary mix of stabilizers and enhancers. Tonbo's Classic++ *Taq* Master Mix has been developed for optimal PCR success. We do not recommend adding 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 reaction mix. If working with final reaction volumes less than 50 µL, scale component volumes accordingly.
3. 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. Refer to the cycling conditions (Table 2) to perform the PCR.

Table 1. Reaction Preparation

Reagent	50 µL reaction	Final Concentration	Notes
Classic++ *Taq* DNA Polymerase Master Mix (2x)	25.0 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
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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