TECHNICAL DATA SHEET

Classic++™ Hot Start *Taq* DNA Polymerase Master Mix

Catalog Numbers: 31-5011-0200R
31-5011-1000R

PRODUCT INFORMATION

Contents: 31-5011-0200R (200 rxns)
Classic++™ Hot Start *Taq* DNA Polymerase Master Mix (2X): 5 x 1.0 mL

31-5011-1000R (1000 rxns)
Classic++™ Hot Start *Taq* DNA Polymerase Master Mix (2X): 25 x 1.0 mL

Use By: 6 months from date of receipt

DESCRIPTION

Classic++™ Hot Start *Taq* DNA Polymerase Master Mix is a premixed, ready-to-use cocktail optimized for efficient amplification from a wide variety of DNA templates. Tonbo's Classic++ Hot Start *Taq* DNA Polymerase is a next generation thermostable DNA polymerase of recombinant origin that possesses 5'→3' polymerase activity, but not 3′→5′ proofreading, exonuclease activity. The enzyme has been further modified by the temperature dependent inclusion of two proprietary Classic++™ Hot Start mAbs (monoclonal antibodies) that act in concert to specifically block polymerase activity below 70°C, allowing for convenient room temperature reaction set up. DNA polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94-95°C for two minutes. The blocking of polymerase activity prior to denaturation of template improves yields by minimizing or eliminating primer dimer formation and non-specific amplification. Convenient and reliable, Classic++ Hot Start *Taq* DNA Polymerase is ideal for standard PCR protocols and has been engineered to provide enhanced speed, yield and specificity over that of standard forms of *Taq* polymerase. In the presence of Tonbo's optimized Master Mix and your primer(s), this hot start enzyme will synthesize double-stranded DNA from a wide variety of single stranded templates.

Tonbo provides a robust, convenient, high performance Master Mix optimized to enhance standard PCR speed, yield and specificity. Classic++ Hot Start *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 hot start polymerase may be used in a wide variety of applications including genotyping, multiplexing, general colony screening and library construction with little to no protocol modification. Use Classic++ Hot Start *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 reagent 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++ Hot Start *Taq* DNA polymerase 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*. Classic++ Hot Start mAbs are of murine origin and are reactive with select epitopes found within recombinant forms of the YT-1 strain of *Thermus acquaticus.*

APPLICATION NOTES

Master Mix: Classic++ Hot Start *Taq* DNA Polymerase Master Mix (2X) contains Classic++ Hot Start *Taq* DNA polymerase, Classic++ Hot Start mAbs, 6 mM $MgCl_2$ and 2 mM dNTPs in a reaction buffer that includes a proprietary mix of stabilizers and enhancers. This Master Mix 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.

Multiplex PCR Suggestions: An annealing temperature gradient from 55°C - 65°C should be generated to empirically determine the temperature that provides the best specificity, which should then be used for subsequent multiplex reactions. We do not recommend using fast cycling conditions for multiplex PCR and suggest starting with a 90 second extension time. If necessary, extension time can be increased for greater yield.

Colony PCR Suggestions: Pick a bacterial colony with a sterile tip and resuspend into the 50 µL reaction mix as described in the Reaction Setup master mix table. From liquid cultures, take 5 µl of overnight culture and add to the 50 µL reaction mix. We recommend increasing the initial denaturation time to 10 minutes.

Direct PCR from Biological Fluids: From mammalian blood or urine, add 2 µL sample to the 50 µL reaction mix, as described under Reaction Setup.

REACTION SETUP / QUICK PROTOCOL

1. Ensure all components are thawed and mixed well. 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 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++ Hot Start 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; For colony PCR, increase denaturation time to 10 minutes
40	95°C	15 seconds	Denaturation
	55°C - 65°C	15 seconds	Anneal
	72°C	1 to 90 seconds	Extension (15 seconds per kb); For multiplex PCR, use 90 seconds

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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