GB-Amp™ 2 x PCR Master mix (with loading dye)

Catalog # P3001 / P3002 / P3003 / P3004

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Introduction

GB-Amp™ 2 x PCR Master mix (with loading dye) contains Taq DNA Polymerase, dNTP, and an optimized buffer system. The amplification can start upon the addition of primer and template, thereby easing PCR setup and improving reproducibility. Cryo-protective agents in the 2× Taq Master Mix enable the resistance to damage from repeated freeze-thaw cycles. Taq DNA Polymerase is a thermostable DNA polymerase that possesses a 5 '→3 ' polymerase activity and a 5 ' flap endonuclease activity. 2× Taq Master Mix is provided with the option of gel loading dyes which enable direct loading PCR products onto agarose gels. The 2 × Taq Master Mix is available in 5, 15 and 50 mL size. The PCR products contain A at the 3'-end and thus can be directly cloned into T -Vectors of TA cloning kits

Content

Components	P3001 1mL	P3002 5mL	P3003 10mL	P3004 50mL
GB-Amp™ 2 x PCR Master mix (with loading dye)	1 mL	5 mL	10 mL	50 mL

Storage

Store at -20℃.

Quality Control

Exonuclease Activity: The product is tested in a reaction containing 10 U of enzyme and 0.6 μg of λ-Hind III. After incubation at 37℃ for 16 hours, there is no visually discernible change to DNA bands determined by agarose gel electrophoresis.

Endonuclease Activity: The product is tested in a reaction containing 10 U of enzyme and 0.6 μg of Supercoiled pBR322. After incubation at 37℃ for 4 hours, there is no visually discernible change to DNA band determined by agarose gel electrophoresis.

Functional Assay: The α-1-antitrypsin gene is amplified for 30 cycles in a 50μL system using 100ng human genomic DNA as template. A single DNA band of 360 bp is detected by agarose gel electrophoresis.

Protocol

1. Recommended general reaction mixture for PCR:

2× Taq Master Mix	25μL
Template DNA*	Variable
Primer 1 (10 μM)	2μL
Primer 2 (10 μM)	2μL
ddH_2O	to 50μL

The recommended amount of DNA template for a 50 μl reaction system is as follows:

Human Genomic DNA	0.1 - 1 μg
Bacterial Genomic DNA	10 - 100 ng
λ DNA	0.5 - 5 ng
Plasmid DNA	0.1 - 10 ng

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2. Thermocycling conditions for a routine PCR:

94℃	5 min (Pre-denaturation)	
94℃	30 sec	} 30 - 35 cycles
55℃*	30 sec	
72℃	60 sec / kb	
72℃	7 min (Final extension)	
4℃	Hold	

*The optimal annealing temperature should be 1-2℃ lower than the T_m of the primers used.

Precaution

Taq DNA Polymerase has significant polymerase activity at room temperature. Therefore, it is recommended to set up PCR reactions on ice. Once set up, these reactions should be immediately subjected to thermal cycling for PCR amplification, in order to reduce nonspecific amplification and get optimal PCR results.

Primers Designing Notes

1. Choose G or C as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer;
3. Avoid hairpin structure at the 3'-end of the primer;
4. T_m of the primers should be within the range of 55℃ - 65℃;
5. Additional tag sequence that are not homologous to target should not be included when calculating Tm of the primers;
6. GC content of the primers should be within the range of 40% - 60%;
7. T_m and GC content of forward and reverse primes should be as similar to each other as possible.

3. Gel Electrophoresis after PCR:

Because the loadingl buffer components required for loading samples onto an agarose gel and to facilitate eletrophoresis have already been added to this 2 x Taq Master Mix, the PCR products can be directly applied to the wells of an agarose gel. For an average minigel, 5-20μL is recommended per well.