15 MAY 2024

AmpFAST 2X PCR SuperMix (5-30 sec/kb; ≤6kb, 1X)

Catalog Number	Size	Concentration
MB202-P040	1 ml x 1 vial	2X

Storage Conditions

Stable for up to 2 years at -20°C

Description

AmpFAST 2X PCR SuperMix was premixed with an optimized concentration of AmpFAST Taq DNA Polymerase, dNTPs, Mg^{2+}, and reaction buffer that could be used in extremely fast PCR experiments as well as massive gene detection requirements, which also performs better amplification functions for GC-rich and complicated secondary structures. For shorter sequence or plasmid samples, it is suggested to use 5 sec/kb elongation speed or less circuits to shorten your PCR reacting time. While for longer sequences (≥3 kb) or complicated samples that usually have lesser PCR products, it is suggested to use 15-30 sec/kb elongation speed or more circuits. Meanwhile, an "A" base at the 3' -end of the PCR products amplified by this enzyme can be directly used in TA cloning.

Kit Content(s)

AmpFAST 2X PCR SuperMix	1 ml x 1 vial

Required materials but not provided

- A compatible PCR instruments
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

Reaction Setup

1. For each 50 µl reaction, assemble the following in a 0.5 ml PCR tube on ice just prior to use:

Component	Volume	Final Conc.
Forward primer, 10 µM	2.5 µl	0.5 µM
Reverse primer, 10 µM	2.5 µl	0.5 µM
AmpFAST 2X PCR SuperMix	25 µl	
DNA template*	X µl	
PCR Grade Water	add to 50µl	-
Total volume	50 µl	

*DNA template: 50-100 ng genomic DNA, 1-30 ng plasmid, or 1-2 µl cDNA from RT-PCR.

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2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in thermal cycler.
3. Process in thermal cycler for 25-35 cycles as follows:

Initial Denaturation	2 mins at 95°C	
Denaturation	15 secs at 95°C	25-35 cycles
Annealing	15 secs at 50-72°C	
Extension	15 sec/kb at 72°C**	
Final extension	5 min at 72°C	

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, templates, and thermal cyclers.

**For simple samples like plasmids, when amplifying DNA fragments up to 3 kb, the extension time can be set at 5 s/kb. For fragments between 3-6 kb, the extension speed ranges from 10 to 15 s/kb. In the case of genomic or cDNA samples, the amplification speed is 15 to 20 s/kb. For samples with high GC content or complex secondary structures, it's advisable to extend the time to 30 s/kb. It's important to note that these are general guidelines for PCR reactions and may need adjustment based on the specific characteristics of the sample, primers, and target fragments. Additionally, the reaction system may require scaling up or down accordingly.

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