FISHER MOLECULAR BIOLOGY

Amplibiotherm PCR 2X Master Mix FS-T-1041-10

Description

Amplibiotherm PCR 2X Master Mix is an optimized premix containing DNA polymerase, dNTPs, MgCl2, KCl and other stabilizers. This product is suitable for conventional PCR amplification. The template can be purified DNA, bacterial colonies/bacteria liquid, crude extract or cDNA, etc. This product can use complex genomic DNA as a template to amplify a target fragment of 5 kb in length or a simple template such as lambda DNA to amplify a target fragment of 10 kb in length. It is suitable for applications such as PCR reaction, colony PCR identification, vector construction and so on. etc.

Kit Contents

Contents	FS-T-1041-10	FS-T-1041-25
Amplibiotherm PCR 2X Master Mix	10 ml	25 ml

1 ml= 40 reactions (Volume of 50 µl)
1 ml= 80 reactions (Volume of 25 µl)

Applications: Conventional PCR

Storage Conditions

Store all components at -20℃ in a non-frost-free freezer.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Amplibiotherm PCR 2X Master Mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1.Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.

2.Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.

Reaction Conditions

Component	25 µl	50 µl	Final Conc.
Amplibiotherm PCR 2X Master Mix	12,5 µl	25 µL	1X
10µM Forward Primer	0.5 µL	1 µL	0.2 µM
10µM Reverse Primer	0.5 µl	1 µL	0.2 µM
Template DNA*	Variable	Variable	<300 ng
Water, RNase-Free	up to 25 µl	up to 50 µl	N/A

*High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed Below

DNA TEMPLATE

DNA	Input Amount
Plants, animals and human gDNA	10 ng~100 ng
E.coli , lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg~10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately

Recommended PCR Program

Step	Temp	Time	Cycles
Pre-denaturation	98°C	45 s	1
Denaturation	98°C	10 s	30
Annealing	55-65°C	30 s	
Extension	72°C	20-30s/kb	
Post-extension	72°C	5min	1
Hold	4-12°C	∞	1

2. Primers :

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1 µM.

3. Denaturation:

98℃ pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation.

Generally, the recommended denaturation condition for low-complexity DNA templates is 98℃, 5-10 s

4. Annealing:

The annealing temperature of Amplibiotherm 2X PCR Mix is usually higher than other PCR polymerases.

Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)℃ for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm. When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

5. Extention:

The recommended extension temperature is 72 ℃ . The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb

6. Cycles:

To obtain enough yield of PCR products, 25-35 cycles are recommended.

For Research Use Only

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36 Terry Drive
Trevose, PA 19048 - USA