

★ Storage

Stable at -20°C in a constant temperature freezer.

★ Contents

- Product Manual
- **amfiXpand** PCR Master Mix
- Water, PCR Certified

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

★ Introduction

amfiXpand PCR Master Mix is ready-to-use mixtures that include all of the reagents needed for PCR. **amfiXpand** PCR Master Mix is composed of a unique enzyme blend containing two thermostable polymerases : Taq DNA polymerase and a thermostable polymerase with proofreading activity. **amfiXpand** PCR Master Mix is designed to amplify fragments up to 5 kb with high yield and fidelity, as well as with high specificity from episomal and genomic DNA. **amfiXpand** PCR Master Mix contains red and yellow loading dyes to allow loading of PCR product directly on a gel after thermal cycling, minimizing pipetting steps and providing easy visualization of sample. The red dye runs in a range between 500bp(2% gels) and 1500bp(0.8% gels) and the yellow dye runs at less than 10bp. **amfiXpand** PCR Master Mix contains a fixed MgCl₂ concentration of 1.75mM. However, higher concentrations may be achieved by adding additional MgCl₂.

★ Components for each reaction

1u/rxn **amfiXpand** Taq DNA Polymerase, Reaction Buffer, 1.75mM MgCl₂, 0.2mM of each dNTP, stabilizers, and red and yellow loading dyes.

★ Usage

The protocol is suggested as a starting point and guideline when using **amfiXpand** PCR Master Mix. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined. We recommend assembling reactions on ice from pre-chilled components.

Note: For multiple reactions with common components, prepare a master mix of the common components for all reactions to reduce pipetting errors.

★ Protocol

1. Thaw **amfiXpand** PCR Master Mix at room temperature. Mix the Master Mix well and then spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

For a 25ul reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	12.5ul	1X
Forward primer, 10uM	0.25-2.5ul	0.1-1.0uM
Reverse primer, 10uM	0.25-2.5ul	0.1-1.0uM
DNA template	1-5ul	≤ 1ug
Nuclease free water to	25ul	N.A

For a 50ul reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	25ul	1X
Forward primer, 10uM	0.5-5ul	0.1-1.0uM
Reverse primer, 10uM	0.5-5ul	0.1-1.0uM
DNA template	1-5ul	≤ 1ug
Nuclease free water to	50ul	N.A

For a 100ul reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	50ul	1X
Forward primer, 10uM	1.0-10ul	0.1-1.0uM
Reverse primer, 10uM	1.0-10ul	0.1-1.0uM
DNA template	1-5ul	≤ 1ug
Nuclease free water to	100ul	N.A

3. Cap reaction tubes and load in the thermal cycler
4. Perform 25-40cycles of PCR amplification

Note : Cycling Parameters

Segment	Number of Cycles	Temperature	Duration
1	1	94°C	4min
2	25-40	94°C	1min
		Primer TM-5°C	1min
		72°C	1min per kb
3	1	72°C	10min

This Cycling Parameter serves as a guideline for PCR amplification. Optimal reaction condition such as PCR cycles, annealing temperature, extension temperature, and predenaturation time and temperature may vary and must be individually determined.

★ Related GenDEPOT Products

Product Name	Cat No
amfiSure Taq DNA Polymerase, 1 unit/ul	P0310
amfiSure PCR Premix, individual 0.2ml tube	P0313
amfiSure PCR Premix, custom order	P0318
amfiXpand Taq DNA Polymerase, 1unit/ul	P0331

★ Tips

CRITICAL OPTIMIZATION PARAMETERS

Template DNA

Usually the template DNA amount is in the range of 0.02-2ng for plasmid or phage DNA and 0.2-2µg for genomic DNA, for a total reaction mixture of 100µl. Higher template DNA amounts usually increase the yield of nonspecific PCR products, but if the fidelity of synthesis is crucial, maximal allowable template DNA quantities in conjunction with limiting number of PCR cycles should be used to increase the percentage of "correct" PCR products. Nearly all routine methods are suitable for template DNA purification. Although even trace amounts of agents used in DNA purification procedures (phenol, EDTA, Proteinase K, etc.) strongly inhibit Taq DNA Polymerase, ethanol precipitation of DNA and repetitive treatments of DNA pellets with 70% ethanol is usually effective in removing traces of contaminants from the DNA sample.

Primers

Guidelines for primer selection:

PCR primers are usually 15-30 nucleotides in length. Longer primers provide sufficient specificity.

The GC content should be 40-60%. The C and G nucleotides should be distributed uniformly within the full length of the primer. More than three G or C nucleotides at the 3'-end of the primer should be avoided, as nonspecific priming may occur.

The primer should not be self-complementary or complementary to any other primer in the reaction mixture, in order to avoid primer-dimer and hairpin formation.

The melting temperature of flanking primers should not differ by more than 5°C, so the GC content and length must be chosen accordingly.

All possible sites of complementarity between primers and the template DNA should be noted.

If primers are degenerate, at least 3 conservative nucleotides must be located at the primer's 3'-end.

Estimation of the melting and annealing temperatures of primer:

If the primer is shorter than 25 nucleotides, the approx. melting temperature (T_m) is calculated using the following formula: $T_m = 4(G + C) + 2(A + T)$

G, C, A, T - number of respective nucleotides in the primer.

Annealing temperature should be approx. 5°C lower than the melting temperature.

If the primer is longer than 25 nucleotides, the melting temperature should be calculated using specialized computer programs where the interactions of adjacent bases, the influence of salt concentration, etc. are evaluated.

Primer concentration

The recommended concentration range is 0.1-1.0µM. Lower primer concentrations may result in lower PCR yield, while higher primer concentrations increase the risk of non-specific amplification.

Reaction overlay

If necessary, the reaction mixture can be overlaid with mineral oil or paraffin (melting temperature 50-60°C) of special PCR grade. One-half of the total reaction volume is usually sufficient.

CYCLING CONDITION

Amplification parameters greatly depend on the template, primers and parameters of a thermal cycler. At GenDEPOT, all functional PCR tests are performed on the GeneAmp[®] PCR System 9700.

Initial DNA Denaturation / Enzyme Activation

The complete denaturation of the DNA template at the start of the PCR reaction is of key importance. Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product. The initial denaturation should be performed over an interval of 1-3min at 95°C if the GC content is 50% or less. This interval should be extended up to 10min for GC-rich templates. If the initial denaturation is no longer than 3min at 95°C, Taq DNA Polymerase can be added into the initial reaction mixture. If longer initial denaturation or a higher temperature is necessary, Taq DNA Polymerase should be added only after the initial denaturation, as the stability of the enzyme dramatically decreases at temperatures over 95°C.

DNA Denaturation

In most conditions, a 0.5-1min DNA denaturation at 95°C is sufficient. For GC-rich DNA templates, this step may be increased to 3-4min. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol or 10% DMSO, 5% formamide or 1.7-2M Betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature must be adjusted accordingly. Additionally, 10% DMSO and 5% formamide inhibit DNA polymerase activity by 50%. Thus, the amount of enzyme should be increased, if these additives are used.

Primer Annealing

In most conditions, the annealing temperature should be 5°C lower than the primer-template melting temperature (T_m). Annealing for 0.5-1min is usually sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments. The annealing temperature must also be adjusted when additives (glycerol, DMSO, formamide or Betaine), which change the melting temperature of the primer-template complex are used.

Extension

The extension step is performed at 72°C. As a general rule, the extension time is 1min per 1kb of the DNA fragment.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected yield of PCR product. If less than 10 copies of the template are present in the reaction, approximately 40 cycles are required. With higher template amounts 25-35 cycles are sufficient.

Final Extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for 5-15min to fill-in the protruding ends of reaction products. If the PCR product is to be cloned into TA vectors, the final extension step can be extended to 30min.