amfiXpand Hot-Start Version DNA Polymerase

P0334

Storage

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

Contents

- Product Manual
- ***amfiXpand Hot-Start Version*** DNA Polymerase, 2u/ul
- ***amfiXpand*** 2X Hot-Start PCR Buffer

Shipping Condition

Ship with ice pack and dry ice.

Introduction

amfiXpand Hot-Start Version DNA Polymerase is designed for hot start PCR, a techinique that enhances the specificity, sensitivity and yield of DNA amplification. In addition, the enzyme provides the convenience of reaction set-up at room temperature. In general, hot start PCR methods reduce or eliminate non-specific primers may bund non-specifically, which often leads to unwanted amplification products and primer-dimers. In order to resolve this problem, ***amfiXpand*** 2X Hot-Start PCR Buffer includes a DNA binding protein that is especially useful at blocking primers at lower temperatures making them unavailable for use by a polymerase. This DNA binding protein effectively blocks DNA synthesis from mis-priming events at lower temperatures. ***amfiXpand Hot-Start Version*** DNA polymerase is an antibody-inactivated DNA polymerase that can be introduced into PCR protocols with no modification to cycling parameters and inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers. The functional activity of the enzyme is restored during a short 4-minute incubation at 95°C.

amfiXpand 2X Hot-Start PCR Buffer

Reaction Buffer, 1.5mM $MgCl_2$ 0.2mM dGTP, 0.2mM dATP, 0.2mM dTTP, 0.2mM dCTP, stabilizers, and yellow loading dye.

Unit Definition

One unit is defined as the amount of the enzyme required to catalize the incor -poration of 10 nmol of dNTPs into a polynucleotide fraction form in 30 mins at 70°C.

Quality Control

PCR, Activity, endonuclease/nickage, Specific performance test.

Protocol

This protocol is proposed as a guideline. Individual reactions require optimization of primer, template and ***amfiXpand Hot-Start Version*** DNA Polymerase concen -trations as well as optimization of cycling temperatures and times.

1. Gently vortex and briefly centrifuge all solutions after thawing. Keep the following components on ice.
2. Add the following components to a thin-wall sitting on ice.

Description	Volume	Final Conc.
2X Hot-Start PCR Buffer	25 ul	1X
Forward primer, 10uM	0.5-5.0 ul	0.1-10. uM
Reverse primer, 10uM	1.5-5.0 ul	0.1-1.0 uM
DNA Template	1.-5 ul	<1 ug
Hot-Start Version DNA Polymerase	0.5-2 ul	1.-4u
Nuclease free water to	50 ul	N.A.

3. Gently Vortex and spin down to collect drops.
4. When using the thermal cycler without a heated lid, overlay the reaction mixture with one-half volume of mineral oil.
5. Perform 25-40 cycles of PCR amplification.

Note: Cycling Parameters.

Segment	Number of cycle	Temperature	Duration
1	1	95 °C	4 min
2	25-40	95 °C	0.5-1 min
		Primer Tm - 5 °C	0.5-1 min
		68 - 72 °C	1 min per kb
3	1	68 - 72 °C	5-10 min

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.

6. Place the PCR tubes in the thermal cycler and start the cycling programs.

Product Selection Guide

Description	Cat No
amfiSure HiFi High Fidelity DNA Polymerase	P0323
amfiSure GC-Rich DNA Polymerase	P0324
amfiSure CloneEasy DNA Polymerase	P0325
amfiSure LQ Long PCR Polymerase	P0326

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Tips

CRITICAL OPTIMIZATION PARAMETERS

Template DNA

Usually the template DNA amount is in the range of 0.02 - 2ng for plasmid of phage DNA and 0.2 - 2ug for genomic DNA, for a total reaction mixture of 100ul. 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 conjuction 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 amount 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 non-specific 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 complementary 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 (Tm) is calculated using the following formula: Tm = 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 temper -rature.
- 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 - 1.0uM. Lower primer concen -trations 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 system9700.

Initial DNA Denaturation/Enzyme Activation

The complete denaturation of the DNA template at the start of the PCR reaction is the key reaction. 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 a interval of 1-3 min at 95°C if the GC content is 50% or less. This interval should be extended up to 10 min for GC-rich templates. If the initial denaturation is no longer than 3 min 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 poly -merase 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 0.5-1 min DNA denaturation at 95°C is sufficient. For GC-rich DNA templates, this step may be increased to 3-4 min. 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 temperature (Tm). Annealing for 0.5-1min is usually sufficient. If non-spe -cific PCR products appear, the annealing temperature should be optimized step -wise 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 extenstion step is performed at 72°C. As a general rule, the extension time is 1 min 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 amount 25-35 cycles are sufficient.

Final Extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for 5-15 min 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 30 min.