amfiSure PCR Master Mix (2X)

P0311

Storage

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

For everyday use an aliquote can be stored at 4°C for up to 2-4 weeks. amfiSure PCR Master Mix is stable for 2-3 days at room temperature. amfiSure PCR Master Mix is stable for 2 years in a constant temperature freezer.

Contents

- Product Manual
- amfiSure PCR Master Mix
- Water, PCR Certified

Shipping Condition

Ship with ice pack.

Introduction

amfiSure PCR Master Mix is ready-to-use mixtures that include all of the reagents needed for PCR. amfiSure PCR Master Mix provides superior yields in both routine and challenging PCR application. Using amfiSure PCR Master Mix in routine and challenging PCR reduces smearing and virtually eliminates unwanted background artifacts. amfiSure PCR Master Mix has been optimized for amplifying DNA template up to 3kb. amfiSure 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% gel) and 1500bp (0.8% gel) and the yellow dye runs at less than 10bp. amfiSure PCR Master Mix contains a fixed MgCl2 concentration of 1.75mM. However, higher concentrations may be achived by adding additional MgCl2.

Components for each reaction

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

Quality Control Assays - Functional Assay

PCR Master Mix is tested for performance in the polymerase chain reaction (PCR) using PCR master mix, 1X, to amplify a 470bp region of the beta-globin gene human genomic DNA. The resulting PCR product is visualized on a Safe-Pinky stained agarose gel.

Protocol

Step 1. Thaw amfiSure 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.

Step 2. Prepare one of the following reaction mixes on ice

Components	Volume	Per 25 ul reaction
PCR Master Mix, 2X	12.5ul	1X
Forward Primer, 10uM	0.25 - 2.5ul	0.1 - 1.0 uM
Reverse Primer, 10uM	0.25 - 2.5ul	0.1 - 1.0 uM
DNA template	1 - 5ul	< 1 ug
Water, PCR certified	upto 25 ul	N.A

Step 3. Cap reaction tubes and load in the thermal cycler.

Step 4. Perform 25-40 cycles of PCR amplification.

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

PCR Reaction Thermocycling

Step	Temp	Time	Cycle
Initial denaturation	96 °C	30-60 sec	1
Denaturation	96 °C	15-30 sec	25-46
Annealing	Primer T_M -5 °C	30-60 sec	
Extension	72 °C	1 min/kb	
Final extension	72 °C	10 mins	1
Hold	4 °C		

Note: An initial denaturation of 30 seconds at 96 °C is sufficient for most amp -licons from pure DNA templates. Longer initial denaturation times can be used (up to 3 minutes) for templates that require it.

Note: the protocol is suggested as a strating point and guidline when using amfiSure PCR Master Mix. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, nay very and must be individually determined. We recommended assembling reaction on ice from pre-chilled components.

Related GenDEPOT Products

Product Name	Cat No
Agarose, Sepro	A0224-050
iVDye 100bp DNA Ladder	V1002-001
1Kb PCR Ranger DNA Marker, 75bp-To-20Kb	D1109-100
SafePinky DNA Gel Staining Solution (10,000X) in water	S1001-100

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Tips

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 distri -buted 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 comple -mentary 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 (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 tem- perature.

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 con- centrations may result in lower PCR yield, while higher primer concentrations increase the risk of non-specific amplification.

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(Tm). 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 tem -plate 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 5-15 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 30min.