

### Storage

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

### Contents

- Product Manual
- **amfiFusion High Fidelity PCR Master Mix (2X)**

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

### Shipping Condition

Ship with ice pack and dry ice.

### Introduction

**amfiFusion High Fidelity PCR Master Mix** combines **amfiXpand** Taq DNA Polymerase with high fidelity, proofreading DNA polymerase. This unique enzyme blend provides superior yields in both routine and challenging PCR. **amfiFusion High Fidelity** DNA Polymerase has 5'-3' exonuclease activity of Taq DNA Polymerase as well as the 3'-5' exonuclease activity of the proofreading DNA polymerase. **amfiFusion High Fidelity** DNA Polymerase increase amplification fidelity up to 6 times over Taq DNA polymerase alone and allows for amplification of longer product size up to 19Kb. **amfiFusion High Fidelity PCR Master Mix** includes a single-strand 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. In addition, **amfiFusion High Fidelity PCR Master Mix** contains red and yellow loading dyes to allow loading PCR product directly on a gel after thermal cycling, minimizing pipetting steps and providing easy visualization of sample.

### Components for each reaction

2 ul/rxn **amfiFusion High Fidelity DNA Polymerase**, 1X Reaction Buffer, 1.0mM MgCl<sub>2</sub>, 0.2mM of each dNTP, Single-strand DNA binding protein and stabilizers.

### Usage

The protocol is suggested as a starting point and guideline when using **amfiFusion High Fidelity PCR Master Mix**. Optimal reaction conditions, such as reaction time, temperature, and amount of template DNA and primers may vary and must be individually determined.

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

### Protocol

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 Master Mix	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
Nuclease free water to	50 ul	N.A.

**Note:** Use DMSO for a difficult GC-rich template. Optimal DMSO concentration must be determined by titration in 1% increments for each primer-template set. See Critical Optimization Parameter for DMSO concentration range recommendations for specific targets.

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
<b>amfiSure HiFi</b> High Fidelity DNA Polymerase	P0323
<b>amfiSure GC-Rich</b> DNA Polymerase	P0324
<b>amfiSure CloneEasy</b> DNA Polymerase	P0325
<b>amfiSure LQ</b> Long PCR Polymerase	P0326