KIAGENE FANAVAR

2X Phusion Master Mix (chimeric Pfu)

Cat. No:
FPLF004.1000
Contents:

Components	
2X Phusion Master Mix	1ml

Kit storage:

⚠ This kit should be stored at -20 °C.
If properly stored, all kit components are stable until the expiration date printed on the label.

Additional Equipment and Reagent required

- Template: genomic DNA, plasmid, phage DNA, cDNA
- Forward and reverse primers
- Agarose
- 1 kb DNA Ladder
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- Water, nuclease-free

Application

The 2X Phusion Master Mix is a ready-to-use mixture of DNA polymerase, salts, magnesium, and dNTPs for efficient PCR amplification. The master mix is ideal for applications where accuracy is important (cloning, sequencing, site directed mutagenesis). The master mix contains the Phusion DNA Polymerase that is a chimeric Pfu which has a DNA binding protein at the N-terminal portion of the gene It catalyzes the polymerization of nucleotides into duplex DNA in the 5'_3' direction, resulting in blunt-ended PCR products without 3'-dA overhangs. Phusion DNA Polymerase exhibits 3'_5' exonuclease (proofreading) activity that enables the polymerase to correct the mis-incorporation of nucleotide, and lacks 5'_3' exonuclease activity. It is suitable for PCR and primer extension reaction that requires high fidelity when the PCR fragment is relatively higher than 3 kb. The enzyme exhibits 3´>5´ proofreading activity, resulting in over 20-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Handling Requirements and Safety Information

⚠ *use RNase-free and DNase-free materials*

⚠ *Do not use any modified Protocols.*

⚠ *Do not pool reagents from different lot numbers.*

⚠ *Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.*

⚠ *After first opening store all bottles in an upright position.*

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

Protocol

1. Prepare reaction by adding the following components in the order listed in the following table.

Components	20 µl rxn	50 µl rxn	Final conc.
2x Phusion Master Mix	10 µl	25 µl	1x
Forward Primer	x µl		0.5 µM
Reverse Primer	x µl		0.5 µM
Template DNA	x µl of 0.01–10 ng plasmid x µl 5–100 ng genomic DNA		
nuclease free Water	Add to 20 µl	add to 50 µl	-

2. Run a thermal cycler program set to the following parameters according to the protocol to be performed.

Cycle	Time	Temp°C
1	4 min	95
25-35 Cycles	30 sec	94
	30 sec	57
	15-30 s/kb	72
1	5 min	72

3. Check 7 µl of PCR products by 0.5 % agarose gel electrophoresis.