KIAGENE FANAVAR

# 2X Pfu Master Mix

## Cat. No:
## FPLF013.1000
## Contents:

| Components | |
|---|---|
| 2x Pfu Master Mix | 1ml |
| Positive Control (Template-Primers) Mixture | 50 µl |

## 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 Pfu 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 Pfu DNA Polymerase, a proofreading DNA polymerase that combines a novel Pyrococcus-like enzyme with a processivity-enhancing domain and universal primer annealing feature.

## 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 Pfu 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<br>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.

## Checking the Activity of Master Mix by positive control (Template-Primers) Mixture

**4.** Prepare reaction by adding
2 µl positive control (Template-Primers) Mixture
+10 µl 2X Pfu Master mix
+8 µl Nuclease Free Water
To a new PCR Microtube and Run a thermal cycler program according to Step 2

**5.** Check 7 µl of PCR products by 0.5 % agarose gel electrophoresis. PCR product with Positive Control should show a band with 750 bp.