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Standard PCR Master Mix 2X (without dye)

Cat # ☐ PCR011 ☐ PCR012 ☐ PCR011-S
Pack Size: ☐ 1.25 mL ☐ 1.25 mLx4 : ☐ 250µl
Storage: -20°C ***Note: Make a working aliquots to minimize the number of freeze-thaw cycles . Mix well prior to use. DO NOT VORTEX***

Introduction

Standard PCR Master Mix 2X (without dye) contains all the components required for a successful polymerase chain reaction, which includes appropriate amounts of dH O, DNA polymerase, dNTPs, MgCl , and reaction buffers for effective amplification. This master mix formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR. The master mix contains polymerase which is a high-fidelity polymerase. In most cases, the error rate is 1 in every 1.3 million base pairs. At 72°C, the DNA Polymerase has a processivity of roughly 1 kb/min. It is compatible with TA cloning

Kit Content:

Components	PCR011	PCR012	PCR011-S
Standard PCR Master Mix 2X (without dye)	1.25 mL (100 PCR reactions)	1.25 mL X 4 (400 PCR reactions)	250 µL (20 PCR reactions)

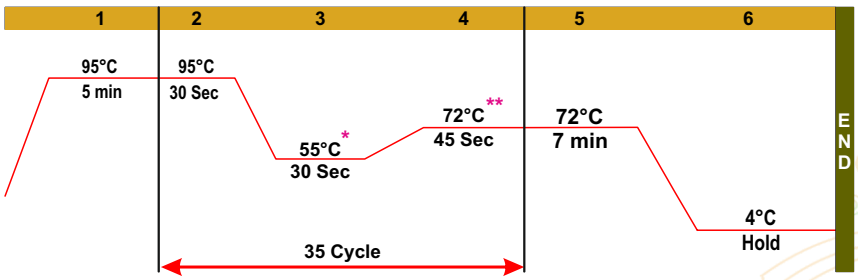
Protocol

1. Gently vortex and briefly centrifuge PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 25 or 50 µL reaction:
3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below

Standard PCR Reaction with Standard PCR Master Mix 2X

Component	For 25µL reaction	For 50µl reaction	Final Concentration
Template (DNA)	Variable	Variable	<1,000 ng
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
Standard PCR Master Mix (2X)	12.5 µl	25 µl	1X
Nuclease-Free Water	to 25 µl	to 50 µl	

General PCR Condition for 1 kb amplification



* Depends on the primer Tm and template used (Need to be optimized)
** Allow approximately 1 minutes for every 1kb to be amplified.

Result

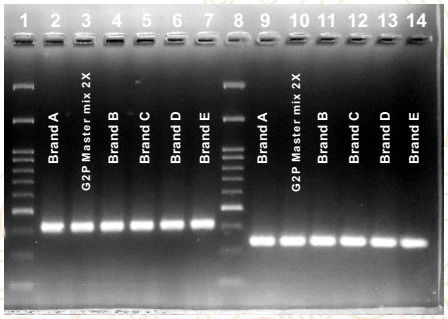


Fig: Different Gene was amplified from plant genome and loaded on 2% agarose gel. Lane 3,10 represent the amplification using Standard PCR Master Mix 2X from Gene to protein Pvt Ltd.

1. No amplification or low yield

- Ensure that the master mix and other components are added correctly and mixed well
- Check that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Increase the amount of template DNA or adjust the template DNA concentration if necessary
- Check the quality of the template DNA, which may be degraded or inhibited
- Increase the extension time or number of cycles in the PCR program
- Make sure the PCR machine is functioning properly
- Use fresh master mix, as they can lose activity over time or after multiple freeze-thaw cycles

2. Non-specific amplification

- Ensure that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Check the specificity of the primers by analyzing the PCR product by gel electrophoresis and sequencing
- Check that the primer concentration is appropriate, as too high or low primer concentrations can cause non-specific amplification
- Reduce the annealing time, as a longer annealing time can increase non-specific amplification
- Increase the extension time, as a shorter extension time can lead to non-specific amplification

- **3. Smearing or multiple bands**

- Ensure that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Check the template DNA concentration, as too much or too little template DNA can cause smearing or multiple bands
- Use a lower annealing temperature, as a higher annealing temperature can cause smearing or multiple bands
- Use a higher extension time or temperature, as a shorter extension time or lower extension temperature can cause smearing or multiple bands

4. PCR inhibition

- Check the purity of the template DNA, as contaminants can inhibit PCR amplification
- Use a different extraction method for the template DNA if necessary
- Dilute the template DNA to reduce PCR inhibition
- Use of additives such as BSA or DMSO may improve PCR amplification in difficult samples