Catalog no. PC-TaqMM02 (2X)
Size: 100 Reactions

Storage: Store at -20°C.
Shipping: Shipped on dry/blue ice.
Shelf Life: 12 Months

Content

- Master Mix (2X)
- Nuclease Free Water (Cat: PC-NFW07)

Description

ProCyto Labs PCR Master Mix is a ready-to-use 2X solution of Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers at optimal concentrations for efficient PCR amplification of DNA templates in the range of 0.2–2kb. The master mix is convenient to use and reduces the chances of contamination by reducing number of pipetting steps. It is helps in quickly setting up the reaction in a minute or two and also in optimizing PCR conditions by changing only either template or primer concentration without adding individual components. The master mix stable for six months. The mix is optimized for efficient amplification of all the templates and results in reproducible PCR.

Applications

- High-throughput PCR
- Routine PCR
- Screening
- Ideal for Cloning

Composition of master mix

- PCR Master Mix (2x)
- Taq DNA polymerase: Supplied in a reaction buffer (pH 8.3)
- dNTPS: dATP, dGTP, dCTP, dTTP (400µM each)
- $MgCl_2$: 1.5mM

Protocol

1. Thaw the master mix and vortex gently and centrifuge.
2. Add the following components in the PCR tube placed on ice.

Reaction Components	25µl Reaction	50µl Reaction	Final Concentration
PCR Master Mix (2X)	12.5 µl	25 µl	-
Forward Primer	0.5 µl - 1 µl	4 µl	10µM
Reverse Primer	0.5 µl - 1 µl	1 µl	10µM
Template DNA	Variable	Variable	10 pg - 10µg
Nuclease Free Water	Make the Volume upto 25µl	Make the Volume upto 50µl	-

3. Gently vortex the samples and spin down.
4. Perform PCR using the recommended thermal cycling conditions outlined below:

PCR Cycles and Conditions

Initial Denaturation - 95°C - 1-3 min - 1 Cycle
*Denaturation - 95°C - 30 Sec
*Annealing - Tm-5 - 30 Sec
*Extension - 72°C - 1 min/kb
Final extension - 72°C - 5-15 min - 1 Cycle

***25-40 cycles of amplification are recommended.**

General Information on PCR Amplification steps:

Initial denaturation

The initial denaturation step ensures to completely denatures the template DNA by heating it to 94°C or higher for effective utilization of the template. An initial denaturation for 1 to 3 min at 95°C is recommended if the GC content of the template is less than 50%. For GC-rich templates, this step can be extended up to 10 min. If a longer initial denaturation step is required, Taq DNA Polymerase should be added after the initial denaturation step to prevent a decrease in its activity.

Denaturation

A DNA denaturation time of 15 to 30 seconds per cycle at 95°C is generally recommended. In the denaturation step, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA template for replication by the thermostable DNA polymerase.

Annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers and is typically 45–68°C. Annealing for 30 to 50 seconds is generally recommended. For non-specific PCR products, annealing temperature can be optimized by temperature gradient PCR with stepwise 1-2°C increments.

Extension

The optimal extension temperature for Taq DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For larger products, the extension time should be extended by 1 min/kb. The extension time depends on the length of the amplicon and the complexity of the template. A final extension of 5 minutes at 68°C is recommended.

Certificate of analysis

No contaminating endonuclease or exonuclease activity is detected.

Functional Assay

PCR Master Mix (2X) was tested for amplification of 1000 bp single copy gene from plasmid DNA.

Quality authorized by:  Anjani Kumar Upadhyay