2X PCR Master Mix (100 Reactions)
Catalogue Number: 28007

Applications:

- Routine PCR amplification of templates
- PCR for post reverse transcription step
- Multiple band detection or genotyping

Reagents supplied:

- 2X PCR Master Mix (1 Vial, 100 Reactions each)
  Sufficient reagent for 100 x 20 µL reactions

Storage Conditions:

2X PCR Master Mix should be stored at -20°C. For everyday use an aliquot can be stored at 4°C for up to 3 months. The 2X Master Mix is stable for multiple freeze-thaw cycles. When stored at the proper temperature this reagent is stable for at least 1 year

Precautions and Disclaimers:

This product is designed for research purposes only. It is not intended for human or diagnostic use.

NORGEN BIOTEK CORP.

ISO 9001:2008 and 13485:2003 Certified Product of CANADA

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Description:

Norgen's 2X PCR Master Mix is a ready-to-use solution that contains components required for PCR amplification including Taq DNA polymerase, dNTPs, reaction buffer, $MgCl_2$, KCl and a PCR enhancer/stabilizer. The user needs only to add template, the primer set and water to the 2X Master Mix to set up the PCR reaction.

Taq DNA Polymerase is a highly thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity and a very low 5´→ 3´ exonuclease activity.

Taq Source:

An *E. coli* strain with a cloned *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Specifications:

- Convenience and time savings
- Cost efficient
- High sensitivity and yield
- Robust amplification

Tech support: techsupport@norgenbiotek.com
Orders: orders@norgenbiotek.com
Toll Free: 1 866 667 4362
Phone: 905-227-8848 Fax: 905 227 1061
www.norgenbiotek.com

Tips for Performing PCR Reactions:

The Polymerase Chain Reaction (PCR) is a powerful method used to amplify specific DNA sequences using multiple cycles of a 3-step process: denaturation, annealing, extension. Successful PCR relies on various factors, and it is important to keep a number of points in mind when performing PCR:

1. Using high quality, purified DNA templates greatly enhances the success of PCR

2. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.

3. There should be designated solutions, tips, tubes, pipettes, etc. for PCR only.

4. Optimize the template amount: up to 1 μg genomic DNA and 100 ng-10 pg for cDNA or plasmid.

Procedure

Reaction Setup Table

PCR Reaction Mixture	20 μL Rxn	50 μL Rxn
2X PCR Master Mix	10 μL	25 μL
Template DNA	1 - 2 μL	1 - 2 μL
Primer F (2.5 μM)	1 μL	1 μL
Primer R (2.5 μM)	1 μL	1 μL
Nuclease-free water	Up to 20 μL	Up to 50 μL

1. Dispense either 10 μL or 25 μL of 2X PCR Master Mix into the PCR tube according to the desired final volume of the PCR reaction (see Reaction Setup Table above).

2. Add template DNA (10 pg – 1 ng for plasmid and 0.1 – 1 μg for genomic DNA) and both forward and reverse primers (2.5 μM of each) to the PCR tube as shown in the Reaction Setup Table.

3. Add nuclease-free water to bring the total volume to either 20 μL or 50 μL.

4. Mix the PCR mixture thoroughly and spin down briefly.

5. Add a drop of mineral oil if the thermocycler is not equipped with a heated lid.

6. Place the PCR tubes into the thermocycler and carry out the PCR according to the Suggested PCR Cycle Conditions shown in the table below.

Suggested PCR Cycle Conditions

PCR Cycle Step	Temperature	Time	No. of cycles
Initial Denaturation	94 - 95°C	2 min	1
Denaturation	94 - 95°C	15 – 30 sec	30 - 40
Annealing	50 - 65°C	15 – 30 sec	
Extension	65 - 72°C	1 minute per kb	
Final Extension	72°C	5 min	1
Hold	4 - 10°C	Indefinitely	1

7. After completion of PCR, a10 μL aliquot of the reaction can be mixed with 2 μL of loading dye (6X) and loaded onto an agarose gel for visual analysis.