# MBT061 2X PCR TaqMixture

## Description

HiMedia's 2X PCR TaqMixture is a premixed, ready-to-use solution containing *Taq* DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient endpoint Polymerase Chain Reaction (PCR) amplification of DNA templates in the range of 0.1 – 3 kb. This pre-mixed formulation saves time to set up the PCR reaction in shorter time and reduce contamination by reduced number of pipetting steps required to set up a PCR. The mix is optimized for efficient and reproducible PCR.

## Product packing

HiMedia's 2X PCR TaqMixture is available in three different packs:

| Product Code | Kit Packing** |
|---|---|
| MBT061-20R | 20R (0.5 mL) |
| MBT061-50R | 50R (1.25 mL) |
| MBT061-100R | 100R (2.5 mL) |

**** The product is supplied with a vial of Molecular Biology Grade Water (ML065)**

## Standard Procedure

1. Thaw the HiMedia's 2X PCR TaqMixture at room temperature.
2. Vortex the master mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.
3. Prepare one of the following reaction mixes on ice:

| Components | Amount to be added for 25 µL | Amount to be added for 50 µL | Final Concentration |
|---|---|---|---|
| 2X PCR TaqMixture | 12.5 µL | 25 µL | 1X |
| Forward primer (10µM) | 0.25 – 2.5 µL | 0.5 – 5.0 µL | 0.1 – 1.0 µM |
| Reverse primer (10µM) | 0.25 – 2.5 µL | 0.5 – 5.0 µL | 0.1 – 1.0 µM |
| Template DNA | 1 – 5 µL | 1 – 5 µL | < 250 ng |
| Molecular Biology Grade Water **(ML065)** | Upto 25 µL | Upto 50 µL | NA |

4. Centrifuge the reactions in a microcentrifuge for 5 seconds.
5. Place the reactions in a thermal cycler that has been preheated to 94°C or 95°C. Perform PCR using standard parameters or using the recommended thermal cycling conditions outlined below:

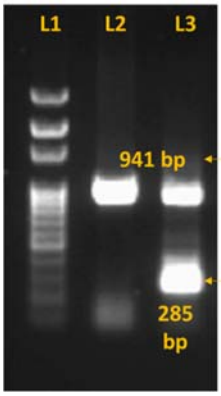
| Step | Temperature (°C) | Time | Number of cycles |
|---|---|---|---|
| Initial denaturation | 94 or 95 | 5-10 mins | 1 |
| Denaturation | 94 or 95 | 30 secs – 1 min | 30-40 |
| Annealing | Tm-5 | 30 secs – 1 min | |
| Extension | 72 | 1 min/kb | |
| Final Extension | 72 | 5 – 15 min | 1 |



ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

**Amplification Data**




| Lane no. | Samples |
|---|---|
| 1 | 100 bp ladder |
| 2 | Amplicon of WSSV DNA (941bp) |
| 3 | Amplicon of WSSV DNA (941bp)with Internal Control (285 bp) |

**Gel image representing amplification of WSSV sample with positive control (941 bp) and internal control (285 bp) using HiMedia's 2X PCR TaqMixture**

**Components of the reaction mixture**

- **Template DNA:** Optimal amounts of template DNA for a 50 µL reaction volume are 1 ng to 1 µg for genomic DNA / phage DNA. Higher amounts of template can generate non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification. All routine DNA isolation kits can be used to prepare the template, e.g. HiPurA™ Blood Genomic DNA Miniprep Purification Kit (MB504), HiPurA™ Bacterial Genomic DNA Purification Kit (MB505). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, may inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

- **Primers:** The recommended concentration range of the PCR primers is 0.1 – 1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products. For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3 – 1 µM.

**General guidelines for PCR amplification**

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension.

- **Initial Denaturation**: The double-stranded DNA melts and forms a single stranded DNA at a high temperature (95°C). A minimum of 5 to 10 minutes initial denaturation step at 95°C is sufficient.

- **Denaturation:** A subsequent denaturation step is between 30 seconds and 1 minute at 95°C.

- **Annealing**: Sequence-specific primers bind to the target sequence on single-stranded DNA, 5°C below the calculated melting temperature (Tm) of the primers and increasing the temperature in increments of 1°C to the annealing temperature. The annealing step is typically 30 seconds to 1 minute.

- **Extension**: *Taq* DNA polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (68-74°C). Approximately 1 minute for every 1 kb of DNA is amplified.

- **Final extension**: A final extension of 5 minutes at 72°C is recommended.

- **Hold**: If the thermal cycler has a hold or soak cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours. In this cycle, the polymerase activity is minimized that might occur at higher temperatures, although this is not usually a problem.

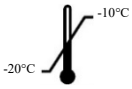
- **Cycle number:** The 3 steps (Denaturation, Annealing and Extension) of PCR are usually repeated between 25 to 30 times in each PCR assay for optimal amplification of desired PCR products. Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

**Quality control**
Each lot of HiMedia's 2X PCR TaqMixture is functionally tested for performance in semi-quantitative PCR.

**Storage and shelf-life**
HiMedia's 2X PCR TaqMixture should be stored at -20°C and is stable for 2 years when stored under proper conditions.

**Technical Assistance**
At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

**Symbols**

| | | | |
|---|---|---|---|
|  | Manufacturer |  | Do not use if package is damaged |
|  | Batch code |  | Temperature limit |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use |
|  | Use-by date (YYYY-MM) |  | Catalogue number |

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HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA. Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email:mb@himedialabs.com Website: www.himedialabs.com