MCLAB
Molecular Cloning Laboratories

User Manual

Version 4.2

Product name: 2x BlueStar™ PCR Master Mix

Cat #: BSPM-100, BSPM-200

Introduction:

2x BlueStar™ PCR Master Mix is a premixed 2x concentrated solution of HoTaq DNA Polymerase, reaction buffer, $MgCl_2$ and dNTPs. The DNA template and primers are simply added for PCR reactions. Worry-free for freeze-thaw associated degradation. The master mix could be stored at 4 °C for up to 6 months without loss in performance. Simply aliquot half of the final volume for each reaction, add template DNA, primers and water, you are ready to go. The premixed blue dye will save your time and you may load the PCR sample after the reaction on to an agarose gel directly, no need for loading dye.
With the high fidelity DNA polymerase in the mix, you could use this master mix not only for the routine clone screening but also for gene cloning directly from the genomic DNA or cDNA reverse transcribed from mRNA.

Features:

- Convenience with loading dye premixed
- Storage at 4 °C, no freeze-thaw
- High sensitivity and yield
- High fidelity and robust amplification

Applications:

- PCR amplification of templates for molecular cloning
- PCR screening for positive clones
- End point PCR for genotyping

Reagents supplied:

2X PCR Master Mix with Blue Dye (1.25 ml and 12.5 ml, up to 100 and 1,000 rxn per vial)

Customer Supplied Reagents and Equipment

- Thermocycler
- Template DNA
- Primers
- Pipettes and tips
- PCR tubes
- Nuclease-free water

Experiment protocol:

1. Aliquot 1/2 of the final volume of 2x BlueStar™ PCR Master Mix into a PCR tube.

2. Add template DNA (10 pg – 1 ng for plasmid and 0.1 – 1 µg for genomic DNA) and both forward and reverse primers (250 nM of each final concentration) to the PCR tube.

3. Add nuclease-free water to bring the total volume to the final volume.

Component	**Volume**	**Volume**
Template DNA	10pg-1ng (plasmid DNA)/0.1-1µg gDNA	10pg-1ng (plasmid DNA)/0.1-1µg gDNA
5' Primer (10 µM)	1 µL	1 µL
3' Primer (10 µM)	1 µL	1 µL
dH_2O	up to 12.5 µL	up to 25 µL
2x BlueStar™ PCR Master Mix	12.5 µL	25 µL
FINAL VOLUME	**25 µL**	**50 µL**

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

5. Place the PCR tubes onto a PCR machine and start the PCR reaction.

6. Load 2 µL or 5 µL of the reaction mixture directly on an agarose gel to check the result after the PCR reaction. Load the rest on an agarose gel if the amplified fragment need to be gel purified for downstream experiments.

PCR Conditions:

Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	10 minute	1
Denaturation	95 °C	30 seconds	25-32
Annealing	5 °C below T_m	30 seconds	
Extension	72 °C	30 seconds/Kb	
Final Extension	72 °C	2 minute	1
	4 °C	Hold	1