MBT108 Hi-SYBr Master Mix (with Hi-Temp DNA polymerase)

Product Name	Product Code	Kit Packing**
Hi-SYBr Master Mix (with Hi-Temp DNA Polymerase)	MBT108-20R	20 reactions (0.5 ml)
	MBT108-50R	50 reactions (1.25 ml)
	MBT108-100R	100 reactions (2.5 ml)
	MBT108-5x100R	5x100 reactions (5x2.5ml)

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

Description:

Hi-SYBr Master Mix (with Hi-Temp DNA Polymerase), supplied in 2X concentration, is a ready to use mix, convenient for real-time PCR. The master mix contains:

- SYBr Green Dye
- Hi-Temp DNA polymerase
- dNTPs
- Assay buffer
- $MgCl_2$

Hi-SYBr Master Mix contains Hi-Temp DNA Polymerase for hot-start PCR, to improve specificity and sensitivity of the PCR by minimizing the formation of non-specific amplification products. The enzyme is active only at high temperatures, where primers no longer bind non-specifically.

Template, primers and nuclease-free water should be added before setting up the PCR reaction.

As the mixture is ready-to-use, the reaction set time is reduced to half.

Principle:

Real-time Polymerase Chain Reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. The presence of dsDNA-binding dye in the Hi-SYBr Master Mix allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens.

ISO 9001-2015 CERTIFIED | WHO GMP CERTIFIED | ISO 13485-2012 CERTIFIED

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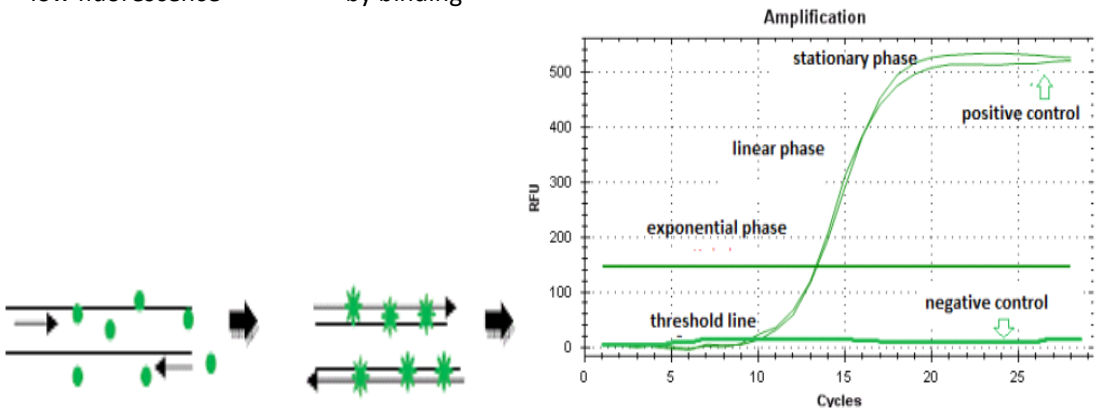
A) Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.

a) Dye in solution emits low fluorescence

b) Emission of the fluorescence by binding

c) Amplification data



SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

Keys : SYBr
Forward primer
Reverse primer
DNA Strand

Standard Procedure:

1. Thaw the Hi-SYBr Master Mix (with Hi-temp DNA polymerase) at room temperature. Vortex the master mix and spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.
2. Prepare the reaction mixture on ice:

For a 20 µl reaction:

Sr.No.	Components	Amount to be added	Final Concentration
1	Hi-SYBr Master Mix (with Hi-Temp DNA Polymerase), 2X	10 µl	1X
2	Upstream primer, 10µM	0.2–2 µl	0.1–1.0µM
3	Downstream primer, 10µM	0.2–2 µl	0.1–1.0µM
4	Template DNA	1-5 µl	<250ng
5	Molecular Biology Grade Water for PCR	Upto 20 µl	-

3. Mix the master mix thoroughly and dispense appropriate volumes into wells of the PCR plate.

4. Add template DNA to individual PCR tubes containing the master mix.

5. Program the real-time PCR machine according to the program outlined.

6. Perform a melting curve analysis of the PCR products.

Recommended PCR program:

1. Initial denaturation : 94°C for 5-10 minutes

2. Cycling Parameters (No. of cycles: 27-35)
   Denaturation : 94°C for 45 seconds
   Annealing : 50-60°C for 30 seconds

3. Plate read after Annealing step then proceed with Melt Curve Stage
4. Melt Curve Analysis as per HiMedia's Insta Q96 Real Time PCR Machine:

95°C : 15 seconds
60°C : 1 minute
95°C : 15 seconds
Increment (°C) : 0.5
Holding time : 10 seconds

NOTE: The user can also set up a melt curve as per existing their PCR instrument.

Quality Control:

Each lot of Hi-SYBr Master Mix is functionally tested for performance in qPCR; free of endo-, exo- deoxyribonuclease, ribonuclease and nicking activities.

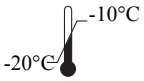
Storage and Shelf-life: The Hi-SYBr Master Mix (with Hi-Temp DNA Polymerase) should be stored at -20°C and kept away from light. The product is stable for 6 months when stored at proper conditions.

Troubleshooting Guide:

Sr. No.	Problem	Solution
1.	Contamination and non-specific amplification	Use Uracil-DNA glycosylase (UDG) along with the master mix, which prevents re-amplification of carry-over PCR products. Use No amplification control and No template control to check for any fluorescent contaminants present. If the fluorescence in No amplification control is greater than No template control, then some fluorescent contaminants are present either in the sample or thermal cycler. Use specific primers to avoid primer-dimers. Practice good laboratory practice in order to avoid contamination.

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.



Storage temperature


Do not use if package is damaged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