MBT074 Hi-SYBr Master Mix (with Taq Polymerase)

Product Name	Product Code	Kit Packing**
Hi-SYBr Master Mix (with Taq Polymerase and No-ROX)	MBT074-0.5ML	0.5 mL (50 reactions)*
	MBT074-1ML	1 mL (100 reactions)*
	MBT074-5ML	5 mL (500 reactions)*

*** For a 20 µL PCR reaction**

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

Description

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

- SYBr Green Dye
- *Taq* polymerase
- dNTPs
- Assay buffer
- $MgCl_2$

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. Real-Time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens. HiMedia's Hi-SYBr Master Mix (with Taq Polymerase) is a 2X concentrated reaction mix that simplifies setup and is pre-blended with reference dye for optimized product performance. The presence of dsDNA-binding dye allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis. SYBR Green dye is a fluorescent intercalating dye which binds to the double stranded DNA and emits a fluorescent signal upon binding. In qPCR, DNA accumulates and fluorescent signal increases proportionally to the DNA concentration. The excitation and emission maxima of SYBR Green dye are at 494 nm and 521 nm, respectively, which are compatible with the use on any Real-Time cycler.

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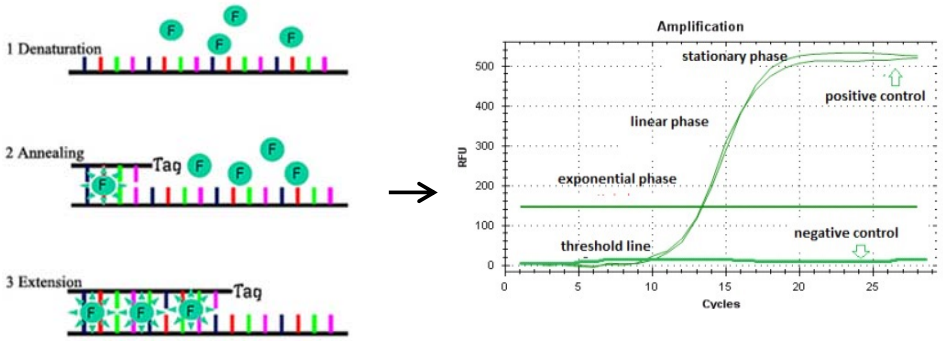
Instrument Compatibility

Different Real-Time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent and internal reference dye to your specific instrument. HiMedia's Hi-SYBr Master Mix (with Taq Polymerase) provides seamless integration on the following Real-Time PCR machines:

1. BioRad's CFX, iCycler iQ™, MyiQ™ and iQ™5
2. Himedia's Insta Q 96 and 48 Series
3. Roche LightCycler 480
4. QIAGEN Rotor-Gene Q
5. Quantabio Q and others

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



The 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.

Standard Procedure:

1. Thaw the Hi-SYBr Master Mix (with Taq Polymerase). 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 (µL)	Final Concentration
1	Hi-SYBr Master Mix (with Taq Polymerase)	10	1X
2	Upstream primer, 10 µM	0.2–2	0.1–1.0 µM
3	Downstream primer, 10 µM	0.2–2	0.1–1.0 µM
4	Template DNA	1-5	< 250 ng
5	Molecular Biology Grade Water for PCR	Upto 20	-

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 or 95 for 5-10 mins	No. of cycles: 1
2.	Denaturation	:	94°C for 10 seconds	No. of cycles: 40
3.	Annealing	:	55 to 60°C for 45 seconds	
4.	Extension (Plate Read)	:	72°C for 30 seconds	
	Channels	:	SYBR	
5.	Melt Curve Analysis as per HiMedia's Insta Q96 Real Time PCR Machine			
	a. 95°C	:	15 seconds	
	b. 60°C	:	1 minute	
	c. 95°C	:	15 seconds	
	d. Increment	:	0.5°C	
	e. On Hold	:	10 seconds	

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

Quality Control

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

Storage and Shelf-life

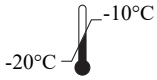
The Hi-SYBr Master Mix (with Taq Polymerase) should be stored between -10°C to -20°C and kept away from light. The product is stable for 1 year when stored at proper conditions.

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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12/2024

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