

MBPCR090

Installation Kit for InstaQ Real Time PCR System

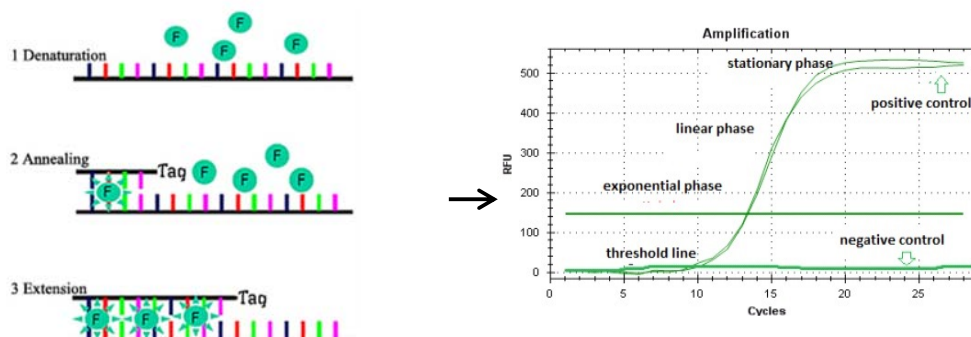
Description

The Installation Kit for Insta Q Real Time PCR System is designed specifically for use during installation of following Real time machines:

- Insta Q96[®] Real Time PCR machine (Product code: LA1012)
- Insta Q48[®] M4 Real Time PCR machine (Product code: LA1023)
- Insta Q48[®] M2 Real Time PCR machine (Product code: LA1024)
- Insta Q96[®] Plus Real Time PCR machine (Product code: LA1073)
- Insta Q96[®] - 6.0 Real Time PCR machine (Product code: LA1074)

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.

Diagrammatic representation of SYBr Green Chemistry 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.

Features:

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results

Storage

The provided kit has a shelf-life of 12 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents

The provided PCR kit contains

Components	Product Code	Reagents provided for 10R (reactions)*
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	150 µL
Primer Mix	DS0580	15 µL
Positive control DNA	DS0581	15 µL
Molecular Biology Grade water for PCR	ML065	500 µL

*For a 25 µL PCR reaction

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Insta Q Real Time PCR System (Product Code: LA1012/LA1073/LA1023/LA1024)
- Barrier Micropipette Tips
- Micropipettes

General Preparation Instructions:

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

A. Protocol for PCR Master Mix Preparation

Perform PCR reactions for each DNA sample as per the following table:

Components	Product Code	Recommended volume to be added per reaction (µL)
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	12.5 µL
Primer Mix (DS0580)	DS0580	1 µL
Positive control DNA (DS0581)	DS0581	1 µL
Molecular Biology Grade water for PCR (ML065)	ML065	Up to 25 µL

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Place the tubes in HiMedia's InstaQ 96/48/48M Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

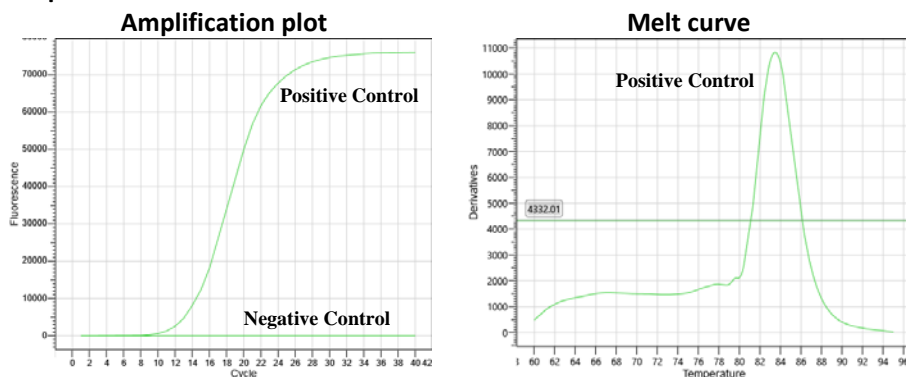
1. Initial denaturation : 94°C for 10 minutes No. of cycles: 1
2. Denaturation : 94°C for 45 seconds
3. Annealing (Plate Read) : 56°C for 30 seconds } No. of cycles: 40
4. Melt Curve Analysis as per HiMedia's Insta Q96 Real-Time PCR Machine
 - 95°C : 15 seconds
 - 60°C : 1 minute
 - 95°C : 15 seconds
 - Increment : 0.5°C
 - On Hold : 10 seconds
5. Hold : 4°C for ∞

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

Quality Control

Each lot of HiMedia's InstaQ Real Time PCR System is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Amplification Data



Sr. No.	Sample	C _t value	T _m (°C)
1	Positive control	11.9	83.5
2	Negative control	N/A	No T _m

Figure: Data representing real-time amplification of Positive Control DNA with T_m values (provided in table)

Precautions

Read the procedure carefully before starting the experiment. Wear protective gloves/protective clothing/eye protection/face protection. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

Each lot of HiMedia's Insta Q Real Time PCR System is tested against predetermined specifications to ensure consistent product quality.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis. 2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

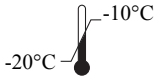
The Installation Kit for Insta Q Real Time PCR System is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

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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