MBT128

Hi-Quanti One Step RT-PCR Kit (Real-Time PCR Based)

Product Name	Product Code	Kit Packing
Hi-Quanti One Step RT-PCR Real Kit	MBT128-10R	10 reactions
	MBT128-25R	25 reactions
	MBT128-50R	50 reactions
	MBT128-2x50R	2 x 50 reactions

Description:-

HiMedia's Hi-Quanti One Step RT-PCR Kit (Real-Time PCR Based) is a convenient one-step formulation, for both cDNA synthesis and PCR amplification in a single tube using gene-specific primers from either total RNA or mRNA. The advantages of one-step real-time RT-PCR is that it is quicker to set up and involves less handling of samples, thereby reducing pipetting errors, contamination and other sources of error. This makes processing multiple RNA samples easy when you are amplifying only a few genes of interest. The MMuLV Reverse Transcriptase enzyme included in the kit consists of a proprietary buffer system that has been optimized for reverse transcription and PCR and includes Mg^{2+} and Deoxy ribonucleotide triphosphates (dNTPs).

Features:

- Efficient, fast RT-PCR in a single tube
- Works efficiently with GC-rich and highly structured RNA templates
- Quick and easy reaction preparation

Hi-Quanti One Step RT-PCR Real Time Kit is provided with:

Components	Reagents provided for 10R	Reagents provided for 25R	Reagents provided for 50R	Reagents provided for 2x50R
SYBr One step RT Buffer	220 µL	550 µL	1.1 mL	2 x 1.1 mL
One Step RT Enzyme Mix	45 µL	110 µL	220 µL	2 x 220 µL
RNA template	Not Provided	Not Provided	Not Provided	Not Provided
Molecular Biology Grade Water	500 µL	1 mL	2 mL	2 x 2mL

ISO 9001-2008 CERTIFIED WHO GMP CERTIFIED ISO 13485-2003 CERTIFIED

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Storage and Stability:
Store the Hi-Quanti One Step RT-PCR Kit (Real-time PCR Based) at –20°C. When stored under recommended conditions, the kit components are stable for 1 year.

Procedure:
1) Add the reagents as follows:

Ingredients	Volume per reaction
SYBr One step RT Buffer	20 µL
One Step RT Enzyme Mix	4 µL
RNA template	X µL
Gene Specific Forward Primer (10 µM)	1 µL
Gene Specific Reverse Primer (10 µM)	1 µL
Molecular Biology Grade Water	Upto 50 µL

2) Gently mix and ensure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed, depending on the thermal cycler used, if necessary.

3) Place the reaction in the preheated thermal cycler programmed as described below. Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.

4) Collect the data and analyze the results.

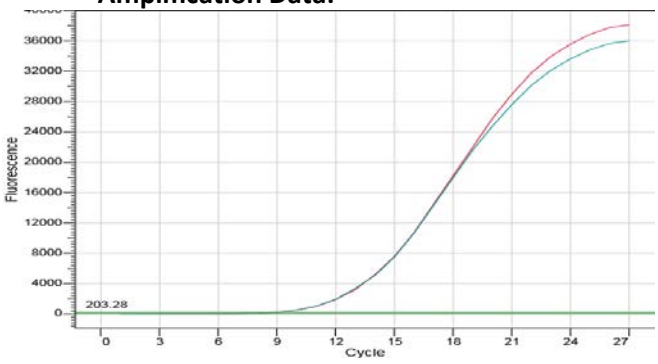
Recommended PCR program:

cDNA Synthesis	:	50°C for 15 minutes	
Initial denaturation	:	95°C for 2 minutes 30 seconds	35 cycles
Denaturation	:	94°C for 30 seconds	
Annealing	:	56-66°C for 30 seconds (Plate Read)	
Melt curve stage	:	Melt Curve Analysis as per HiMedia's Insta Q96 Real Time PCR Machine	
		95°C for 15 seconds	
		60°C for 1 minute	
		95°C for 15 seconds	
		Increment for 0.5°C	
		Holding time for 10 seconds	

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

Quality control:
Detected free of RNases, endonuclease and exonuclease activities.

Amplification Data:



Sr. No.	Sample	C_t value
1	Negative control	N/A
2	Representative data of RT-PCR using Chikungunya sample	10.36
3		10.29

Image representing real-time amplification data of Chikungunya samples with C_t values (provided in table)

Troubleshooting Guide:

Sr.No.	Problem	Possible cause	Possible solution
1	No amplification product	RNase contamination	Maintain aseptic conditions; add RNase inhibitor
		Not enough starting template RNA	Increase the concentration of template RNA
		RNA has been damaged or degraded	Replace RNA, if necessary
		RT inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. **Note:** Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine
		Annealing temperature is too high	Decrease temperature as necessary
		Extension time is too short	Set extension time for at least 60 seconds per kb of target length
		Cycle number is too low	Increase cycle number
2	Low specificity	Reaction conditions not optimal	• Optimize magnesium concentration • Optimize the primer • Optimize the annealing temperature and extension time • Increase temperature of RT reaction to 60°C
		Oligo (dT) or random primers used for first-strand synthesis	Use only gene-specific primers
3	Unexpected bands after electrophoretic analysis	Contamination by genomic DNA	• Pretreat RNA with DNase I
		Nonspecific annealing of primers	• Vary the annealing temperature • Optimize the magnesium concentration for each template and primer combination
		Primers formed dimers	Design primers without complementary sequences at the 3´ ends

Safety Information

The Hi-Quanti One Step RT PCR Kit (Real Time PCR Based) 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.

Consult instructions for use

Do not use if package is damaged

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