MBT138 HiScript Two Step RT-PCR Kit

Product Name	Product Code	Kit Packing
HiScript Two Step RT-PCR Kit	MBT138-10R	10 reactions
	MBT138-25R	25 reactions
	MBT138-50R	50 reactions

Description:

HiScript Two Step RT-PCR Kit is designed for reverse transcription where cDNA (complementary DNA) is synthesized *in vitro* from an mRNA template by an enzyme that has reverse transcriptase activity followed by PCR amplification.

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV RTase) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis. This step is very important in order to perform PCR since DNA polymerase can act only on DNA templates. The resulting cDNA is single-stranded and this process is called reverse transcription (RT) or first strand cDNA synthesis.

The HiScript Two Step RT-PCR Kit is designed for simplicity and convenience in carrying out RT-PCR followed by PCR amplification.

Owing to its good amplification efficiency, specificity and stability, it can greatly improve the sensitivity. The product is designed for convenient, good sensitivity for two step RT-PCR reaction. Its unique enzyme and tailor-made buffer can ensure the efficiency and accuracy of reverse transcription PCR reaction.

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
RT Buffer	110 µl	260 µl	520 µl
10X Solution	55 µl	130 µl	260 µl
M-MuLV Reverse Transcriptase	25 µl	55 µl	105 µl
2X PCR Master Mix **(MBT061)**	260 µl	650 µl	1.3 ml

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

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Gene Specific Forward Primer (10 µM)	Not Provided	Not Provided	Not Provided
Gene Specific Reverse Primer (10 µM)	Not Provided	Not Provided	Not Provided
RNA template	Not Provided	Not Provided	Not Provided
Molecular Biology Grade Water	500 µl	1 ml	2ml

Storage and Stability

Store the HiScript Two Step RT-PCR Kit at –20°C in a constant-temperature freezer. When stored under these conditions, the kit components are stable for 1 year.

Procedure

1) Add the reagents as follows:

Ingredients	Volume per reaction
Reverse Transcription Buffer	10 µl
10X solution	5µl
M-MuLV Reverse Transcriptase	2µl
RNA template	5µl
Molecular Biology Grade Water	Upto 25 µl

2) Gently mix and ensure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

3) For preparation of cDNA, incubate the complete reaction mix for:
   15 minutes at 50°C,
   Hold at 4°C (optional)

4) The cDNA can be further used to perform PCR assay.

Ingredients	Volume per reaction
2X PCR Master Mix **(MBT061)**	25 µl
Gene Specific Forward Primer (10 µM)	2µl
Gene Specific Reverse Primer (10 µM)	2µl
cDNA (as a template)	1-2µl
Molecular Biology Grade Water	Upto 50 µl

Recommended PCR program:

- Initial denaturation : 94°C for 5 minutes
- Cycling Parameters (No. of cycles: 40)

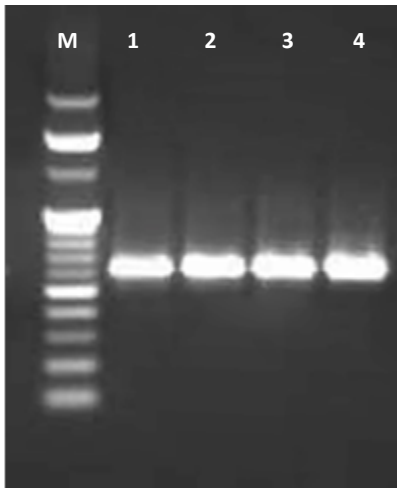
  Denaturation : 94°C for 30 seconds

  Annealing : 55-66°C for 30 seconds

  Extension : 72°C for 30 seconds

- Final Extension : 72°C for 5 minutes

Representative data of PCR of amplification using MBT138



Lane M: 100 bp DNA Ladder; **Lane 1 to 4**: RT-PCR Product

Quality control:

Detected free of RNases, endonuclease and exonuclease activities.

Troubleshooting Guide:

Sr.No.	Problem	Possible cause	Possible solution
1	No amplification product	No cDNA synthesis (temperature too high)	For the cDNA synthesis step, incubate <50°C.
		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
		Primers formed dimers	Design primers without complementary sequences at the 3´ ends

Safety Information

The HiScript Two Step RT-PCR Kit 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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