MBT095 **HiScript One Step RT-PCR Kit**

Product Name	Product Code	Kit Packing
HiScript One Step RT-PCR Kit	MBT095-10R	10 reactions
	MBT095-25R	25 reactions
	MBT095-100R	100 reactions

Description:

HiScript One Step RT-PCR Kit is designed for sensitive, high-fidelity end-point detection and analysis of RNA molecules by RT-PCR. Using this convenient one-step formulation, performing both cDNA synthesis and PCR amplification in a single tube using gene-specific primers and target RNAs from either total RNA or mRNA has become very easy. The system uses a mixture of M-MuLV reverse transcriptase and Hi-Proof DNA Polymerase for enhanced RT-PCR yields and fidelity, as well as the detection of longer templates. The amount of starting material can range from 1 pg to 1 µg of total RNA.

The system consists of two major components: Reverse transcriptase Enzyme Mix and 2X Reaction Mixture. The enzyme can synthesize cDNA at a temperature range of 45–60°C, providing increased specificity, and higher yields of cDNA.

The Enzyme Mix included in the kit consists of a proprietary buffer system that has been optimized for reverse transcription and PCR, and includes Mg^{2+}, deoxyribonucleotide triphosphates (dNTPs). A tube of 50 mM $MgCl_2$ is included in the kit for further optimization of the Mg^{2+} concentration.

Note: This kit has been optimized for end-point RT-PCR (Semi-quantitative) and for Real-time PCR.

ISO 9001-2008 CERTIFIED

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HiScript One Step RT-PCR Kit is provided with:

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 100R (reactions)
2X Reaction Mixture	260 µl	650 µl	2.6 ml
Reverse Transcriptase Enzyme Mix	10 µl	25 µl	110 µl
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 (10 pg to 1 µg total RNA)	Not Provided	Not Provided	Not Provided
Molecular Biology Grade Water	500 µl	1 ml	2.5 ml

Storage and Stability:

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

Procedure:

1) Add the reagents as follows:

Ingredients	Volume per reaction
2X Reaction Mixture	25 µl
Reverse Transcriptase Enzyme Mix	1 µl
RNA template (10 pg to 1 µg RNA)	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 make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed. Depending on the thermal cycler used, overlay with mineral oil if necessary.

3) Place the reaction in the preheated thermal cycler programmed as described below. Collect the data and analyze the results.

4) Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.

A. cDNA Synthesis and Pre-denaturation:

Perform 1 cycle of: 45-60°C for 15-30 minutes
94°C for 2 minutes

B: PCR amplification:

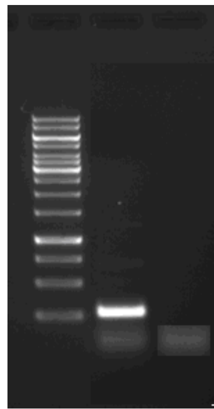
Perform 40 cycles of: Denature, 94°C for 15 seconds
Anneal, 55-66°C for 30 seconds
Extend, 68°C for 1 minute/kb

C: Final extension:

1 cycle of 68°C for 5 minutes

Observations and Results:

1 2 3



Lane 1: 1kb DNA Ladder
Lane 2: RT-PCR Product (Test)
Lane 3: RT-PCR Product

Representative data of PCR using cDNA synthesized by MBT095

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 <55°C
		RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
		Not enough starting template RNA	Increase the concentration of template RNA; use 100 ng to 1 µg of total 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.

Sr.No.	Problem	Possible cause	Possible solution
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.
			Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA.
		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.

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.

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