MBT076 Hi-cDNA Synthesis Kit

Product Name	Product Code	Kit Packing
Hi-cDNA Synthesis Kit	MBT076-10R	10 reactions
	MBT076-25R	25 reactions
	MBT076-100R	100 reactions

Description:

Hi-cDNA Synthesis 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. Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV RT) is an RNA-dependent DNA polymerase that is 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 kit contains Random Hexamer, Oligo (dT) and Random Hexamer : Oligo(dT) Mix along with Ribonuclease Inhibitor.

Random Hexamers are commonly used for priming single-stranded DNA or RNA for extension by DNA polymerases or reverse transcriptases.

Oligo(dT) is single-stranded sequence of deoxythymine (dT), used for priming reactions catalyzed by reverse transcriptase. The transcript is primed in the poly(A) tail of mRNA molecules.

Ribonuclease Inhibitor are commonly used as a precautionary measure in cDNA synthesis to inhibit ribonucleases (RNases) that can sometimes co-purify with isolated RNA and compromise downstream applications.

Hi- cDNA Synthesis Kit is provided with:

Components	Product Code	Reagents provided for (reactions)		
		10R	25R	100R
RT Buffer for M-MuLV	DS2163	50 µL	125 µL	500 µL
10X Solution for M-MuLV	DS2164	25 µL	62.5 µL	250 µL
M-MuLV Reverse Transcriptase (RNase H-)	DS2162	12 µL	30 µL	120 µL
Ribonuclease Inhibitor	DS0921	7 µL	17.5 µL	70 µL
Random Hexamer	DS0146	15 µL	37.5 µL	150 µL
Oligo (dT)	DS0145	15 µL	37.5 µL	150 µL
Random Hexamer : Oligo(dT) Mix	DS0922	25 µL	62.5 µL	250 µL
10 mM dNTP Mix	MBT078	25 µL	62.5 µL	250 µL
Molecular Biology Grade Water for PCR	ML065	500 µL	1.25 mL	5 mL

Storage and Stability

Store the Hi-cDNA synthesis Kit at -10°C to -20°C in a constant-temperature freezer. When stored under these conditions, the kit components are stable for 15 months.

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

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Materials needed but not provided:

- Thermal cycler
- PCR tubes (Product code: PW1255) or PCR Strips (Product code: PR17, PR22, PR23) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

Procedure

1. Add the reagents as follows:

Ingredients	Code	Volume per reaction		
		Random Hexamer	Oligo(dT)	Random Hexamer:Oligo(dT) Mix
Random Hexamer*	DS0146	1 µL	-	-
Oligo(dT)*	DS0145	-	1 µL	-
Random Hexamer : Oligo(dT) Mix*	DS0922	-	-	2 µL
RNA template	-	5 ng to 5 µg		
Molecular Biology Grade Water for PCR	ML065	Up to 10 µL		

*Alternatively, one can use a gene-specific reverse transcription primer.

2. Incubate for 5 min at 65°C, then cool immediately on Ice.

3. Prepare the reaction mixture in a total volume of 20 µL.

Ingredients	Code	Volume per reaction
Template RNA Primer Mixture (from step 2)		10 µL
RT Buffer for M-MuLV	DS2163	4 µL
10X Solution for M-MuLV	DS2164	2 µL
M-MuLV Reverse Transcriptase (RNase H-)	DS2162	1 µL
Ribonuclease Inhibitor	DS0921	0.5 µL
10 mM dNTP mix	MBT078	2 µL
Molecular Biology Grade Water for PCR	ML065	Up to 20 µL

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

5. For preparation of cDNA using, incubate the complete reaction mix as follows:
   a. **For preparation of cDNA using, incubate the complete reaction mix using Random Hexamer**

Random Hexamer	No. of cycles
25°C for 5 minutes	1 cycle
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

OR

   b. **For preparation of cDNA using, incubate the complete reaction mix using For Oligo(dT)**

Oligo(dT)	No. of cycles
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

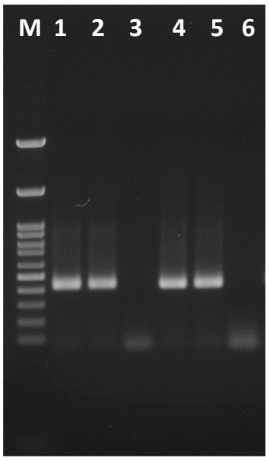
OR

c. For preparation of cDNA using, incubate the complete reaction mix using Random Hexamer : Oligo(dT) Mix

Random Hexamer:Oligo(dT) Mix	No. of cycles
25°C for 5 minutes	1 cycle
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

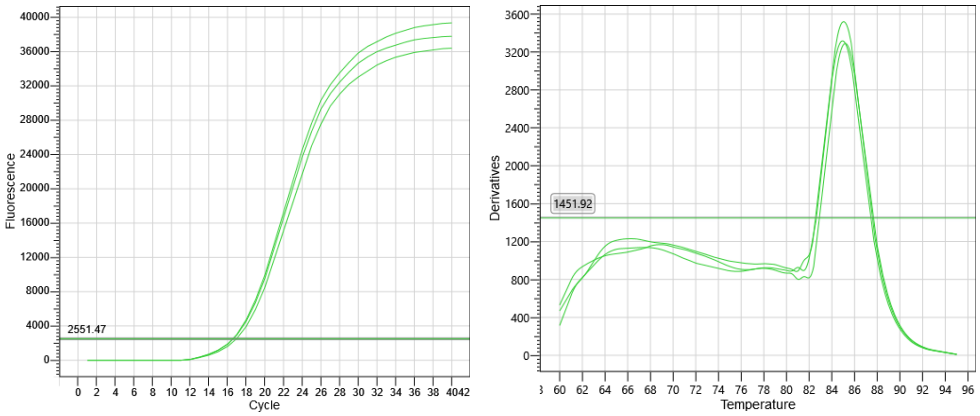
6. The cDNA can be further used to perform conventional or Real-Time PCR assay.

Amplification Data



Lane	Sample
M	100bp DNA ladder
1,2	Amplicon obtained using Random Hexamer
4,5	Amplicon obtained using Oligo(dT)
3,6	Negative controls

Representative data of Semi-quantitative PCR of cDNA synthesized using Hi-cDNA Synthesis Kit (MBT076) after amplification



Ct value	Tm
17.01	85.2
16.64	85
16.7	84.9

Representative data of Real-time SYBr based PCR of cDNA synthesized using Random hexamer : Oligo (dT) mix of Hi-cDNA Synthesis Kit (MBT076) after amplification

Quality control

Detected free of RNases, endonuclease and exonuclease activities.

Warning

Not for Medicinal Use

Precautions

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

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
		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
		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 Hi-cDNA Synthesis Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

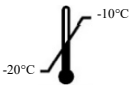
Disposal

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.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBT076
Rev.No.:16
Date of Issue: 2025-04

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