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iScript™ All-in-One RT Master Mix (5X)

Catalog Number: D043-1, D043-2

Table 1. Kit Components and Storage

Kit Component	D043-1 (25 rxns)	D043-2 (100 rxns)	Storage	Stability
5× All-in-One Master Mix	50 µL	200 µL	-20°C in a non-frost-free freezer	The product is stable for one year when stored as directed.
Nuclease-free H_2O	250 µL	1 mL		

Product Description

iScript™ 5X All-In-One RT Master Mix is a convenient, ready-to-use formulation of all the reagents necessary for first-strand cDNA synthesis with the exception of the template. This optimized, 5X All-In-One RT Master Mix contains novel iScript™ IV Reverse Transcriptase (RT), RNase Inhibitor, dNTPs and a finely balanced ratio of Oligo$(dT)_{20}$ and Random Primers. Oligo$(dT)_{20}$ anneals selectively to the poly(A) tail of mRNAs. Random Primers do not require the presence of poly(A) and they are utilized for the transcription of mRNA 5'-end regions. The first-strand cDNA can be directly used as a template in PCR.

iScript™ IV Reverse Transcriptase (RT) is a novel recombinant reverse transcriptase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. The iScript™ IV Reverse Transcriptase is engineered to work under high temperatures (50-55°C), which can further facilitate to resolve the secondary structures and high GC problems of RNA. Besides, the iScript™ IV RT is significantly improved in inhibitor resistance, processivity, and reaction speed. iScript™ IV RT is designed to provide reliable, consistent, and fast cDNA synthesis in the presence of inhibitors found in a wide variety of samples.

Applications

- cDNA synthesis for PCR.
- Construction of cDNA libraries.
- Generation of probes for hybridization.

Primer Information

Oligo$(dT)_{20}$ are oligonucleotides that anneal to the 3'-Poly(A) tail of mRNAs. Therefore, the utility of Oligo$(dT)_{20}$ is restricted to case scenarios where only mRNA or total RNA templates with 3'-Poly(A) tails are used for cDNA synthesis. On the other hand, since Random Primers anneal at non-specific sites within RNA template(s), they can be used generically for all forms of RNA as template for cDNA synthesis.

General Protocol for First-Strand cDNA Synthesis

RT reactions should be assembled in a RNase-free environment. The use of clean pipettes designated for PCR and aerosol resistant barrier tips are recommended.

1. Thaw template RNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare the following reaction mixture in a tube on ice:

Component	Volume	Final Concentration
Total RNA or poly(A)$^+$RNA	x µL	1 ng-2 µg total RNA or 10 pg-500 ng mRNA
5× All-in-One Master Mix	2 µL	1×
Nuclease-free H_2O	to 10 µL	

3. Mix the components well and collect by brief centrifugation. Incubate the tube at 25°C for 10 mins.
4. Incubate the tube at 50°C for 50 minutes.
5. Stop the reaction by heating at 85°C for 5 minutes. Chill on ice. The newly synthesized first-strand cDNA can be used directly for PCR.

Notes:

1. Isolation of poly(A)+RNA from total RNA is not mandatory. However, doing so may improve the yield and purity of the final product.
2. In most cases, cDNA synthesized with this enzyme can be directly used as a template for most polymerase chain reaction (PCR), without further purification. Generally, dilute the final reaction mix for 10 times with water. Use 1-2 µl of the diluted reaction mix for each PCR reaction.
3. To remove RNA complementary to the cDNA, add 1 µl (2 U) of *E. coli* RNase H and incubate at 37°C for 20 mins.
4. RNA sample must be free of contaminating genomic DNA.
5. The synthesized cDNA should be stored at -20°C.