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iScript™ All-in-One RT Master Mix (gDNA Removal)

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 RT Master Mix	100 µL	400 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 for first-strand cDNA synthesis, including genomic DNA (gDNA) removal, all in one tube. This powerful formulation addresses the common challenge of genomic DNA contamination, ensuring accurate RNA detection without compromising reverse transcription or cDNA synthesis. cDNA synthesis will be simple, reliable, and reproducible.

The optimized 5X RT Master Mix includes ABP novel iScript™ IV Reverse Transcriptase (RT), RNase Inhibitor, temperature-sensitive DNase, dNTPs, and a carefully balanced mix of Oligo(dT)s and Random Primers. The iScript™ IV Reverse Transcriptase features genetic modifications that abolish RNase H activity to achieve thermal stability, allowing the RNA template to remain intact during reverse transcription for enhanced cDNA yield and integrity. By eliminating the need for multiple separate reagents, this all-in-one mix offers a straightforward, reliable, and reproducible workflow that consistently delivers high-quality cDNA, even from challenging or low-quality RNA samples—ideal for a wide range of downstream molecular biology applications.

Special Features

- Easy one-step setup reduces pipetting and sample handling.
- Removes contaminating gDNA with temperature-sensitive DNase.
- High-quality cDNA yield for downstream applications.

Applications

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

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)$^+$mRNA	x µL	1 ng-2 µg total RNA or 10 pg-500 ng mRNA
5×All-in-One RT Master Mix	4 µL	1×
Nuclease-free H_2O	to 20 µL	

3. Mix the components well and collect by brief centrifugation.
4. Incubate the tube at 37°C for 5 minutes, followed by 50°C for 15 minutes.
5. Stop the reaction by heating at 90°C for 1 minute. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

Notes:

1. Both poly(A)+mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A)+mRNA may give higher yields and improve purity of final products.
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. For more efficient gDNA removal, increase the length of 37°C incubation from recommended 5 minutes to 30 minutes.
6. The synthesized cDNA should be stored at -20°C.