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iScript™ IV First-Strand cDNA Synthesis Kit with dsDNase

Catalog Number: D042-1, D042-2

Table 1. Kit Components and Storage

Kit Component	D042-1 (25 rxns)	D042-2 (100 rxns)	Storage	Stability
dsDNase	25 µL	100 µL	-20°C in a non-frost-free freezer	The product is stable for one year when stored as directed.
dsDNase Buffer (8X)	50 µL	200 µL		
RT Enzyme Mix (10X)	50 µL	200 µL		
RT Reaction Mix (2X)	250 µL	1 mL		
Nuclease-free H_2O	250 µL	1 mL		

Product Description

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.

iScript™ IV First-Strand cDNA Synthesis Kit with dsDNase is a proprietary mixture of all materials required for first-strand cDNA synthesis. dsDNase is used to remove contaminating gDNA from the sample prior reverse transcription. It allows for dramatically simplified workflow which combines genomic DNA elimination and cDNA synthesis into one-tube procedure. This optimized RT Reaction Mix (2X) contains dNTPs, and a balanced concentration for Oligo$(dT)_{20}$ and Random Primers. The RT Enzyme Mix contains iScript™ IV Reverse Transcriptase and RNase Inhibitor. 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.

Applications

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

Product Specifications

- **Storage Buffer:** 20 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.01% (v/v) NP-40, and 50% (v/v) glycerol.
- **Unit Definition:** One unit is defined as the amount of enzyme required to incorporate 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)/ oligo$(dT)_{18}$ as a template/primer.
- **Unit Reaction Conditions:** 50 mM Tris-HCl (pH 8.3), 4 mM $MgCl_2$, 10 mM DTT, 50 mM KCl, 0.5 mM dTTP, 0.4 MBq/mL [^{3}H]-dTTP, 0.4 mM poly(A)/oligo$(dT)_{18}$ and enzyme in 20 µl for 10 min at 37°C.

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
dsDNase	1 µL	
dsDNase Buffer (8X)	1 µL	1×
Nuclease-free H_2O	to 8 µL	

3. Gently mix the samples and spin down.
4. Incubate for 2 min at 37°C in the preheated thermomixer or water bath.
5. Add 10 µL RT Reaction Mix (2X), gently mix the samples. Heat mixture to 65°C for 5 min and incubate on ice for at least 1 min. Collect all components by a brief centrifugation.
6. Add 2 µL RT Enzyme Mix (10X), mix thoroughly and carefully by vortexing for 3 -5 seconds. Centrifuge briefly to collect the contents of the tube.
7. Incubate the tube at 25°C for 10 minutes, then at 50°C for 50 minutes.
8. 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. Unlike the oligo(dT) priming, which usually requires no optimization, the ratio of a random primer to RNA is critical in terms of the average length of cDNA synthesized in the reaction. Increasing the ratio of random primer/RNA will result in higher yield of shorter (~500bp) cDNA, whereas decreasing this ratio will produce longer products.
6. The synthesized cDNA should be stored at -20°C.