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iScript™ IV Reverse Transcriptase

Catalog Number: D040-1, D040-2

Table 1. Kit Components and Storage

Kit Component	D040-1 (5,000 units)	D040-2 (20,000 units)	Storage	Stability
iScript™ IV RTase (200 U/µL)	25 µL	100 µL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.
5× RT Buffer	150 µL	600 µL		

Product Description

iScript™ IV Reverse Transcriptase (RT) is a novel MMLV mutant with superior robustness and reliability in RT reactions. It exhibits much higher efficiency in the first strand cDNA synthesis from RNA templates with secondary structures and high GC contents. The iScript™ IV Reverse Transcriptase is engineered to work under high temperatures (50°C-55°C), which can further facilitate to resolve the secondary structures and high GC problems of RNA. Besides, the iScript™ IV Reverse Transcriptase 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.

The enzyme is available in 5,000 and 20,000 unit sizes at a concentration of 200 U/µL. The enzyme is supplied with a 5× Reaction Buffer.

Applications

- RT-PCR.
- Real Time RT-PCR.
- cDNA synthesis.

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 of iScript™ IV RT is 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. (End user supplies Oligo(dT), Random Primers, dNTP and RNase-free H_2O.)
2. Prepare the following reaction mixture in a tube on ice:

Component	20 µL rxn	Final Concentration
Total RNA or poly(A)$^+$RNA	x µL	10 pg-5 µg total RNA or 10 pg-500 ng mRNA
Oligo d(T)$_{20}$ primer (50 µM) or Random primer (50 µM) or Gene-specific primer (2 µM)	1 µL 1 µL 1 µL	2.5 µM 2.5 µM 0.1 µM
dNTP (10 mM)	1 µL	500 µM
5× RT Buffer	4 µL	1×
RNase inhibitor (40 U/µL)	0.5 µL	20 U/rxn
iScript™ IV RTase (200 U/µL)	1 µL	200 U
RNase-free H_2O	to 20 µL	

3. Mix thoroughly and carefully by vortexing for 3 -5 seconds. Centrifuge briefly to collect the contents of the tube, and incubate at 25°C for 5 minutes if random primer is used. Omit this step if Oligo(dT)$_{20}$ primer or sequence-specific primer are used.
4. Incubate at 50°C for 60 minutes.
5. Stop the reaction by heating at 85°C for 10 minutes. Chill on ice. The 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. RNA sample must be free of contaminating genomic DNA.
4. 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.
5. The synthesized cDNA should be stored at -20°C.