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iScript™ IV One-Step RT-PCR Kit

Catalog Number: D044-1, D044-2

Table 1. Kit Components and Storage

Kit Component	D044-1 (100 rxns)	D044-2 (200 rxns)	Storage	Stability
RT-PCR Enzyme Mix (10X)	200 µL	400 µL	-20°C in a non-frost-free freezer	The product is stable for one year when stored as directed.
RT-PCR Reaction Mix (5X)	400 µL	800 µL		
Nuclease-free H_2O	1 mL	2×1 mL		

Product Description

iScript™ IV One-Step RT-PCR Kit contains all necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube. RT-PCR Enzyme Mix (10X) contains iScript™ IV Reverse Transcriptase (RT), Hot-Start Taq DNA Polymerase and RNase Inhibitor for highly sensitive and specific RT-PCR using any RNA template. Our proprietary RT-PCR Reaction Mix (5X) contains stabilizers and enhancers that optimize the two reactions in a “single step”. Together with a specially formulated RT-PCR Reaction Mix, this One-Step RT-PCR Kit offers the end-users an efficient and reliable alternative to conventional “two-step” sequential RT-PCR.

Applications

- Gene-expression analysis.
- Transcription analysis.
- Gene cloning.
- Virus detection and quantification.

General Protocol

RT-PCR reactions should be assembled in a nuclease-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 PCR 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× RT-PCR Reaction Mix	4 µL	1×
10× RT-PCR Enzyme Mix	2 µL	1×
Forward Primer (10 µM)	1 µL	500 nM
Reverse Primer (10 µM)	1 µL	500 nM
Nuclease-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.
4. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification automatically.

Steps	Temperature	Duration	Cycle
cDNA Synthesis	50^{o}C	30 min	1
Initial Denaturation	95^{o}C	3 min	1
Denaturation	95^{o}C	15 sec	30-35
Annealing*	55^{o}C	30 sec	
Extension	72^{o}C	1 kb/min	
Final Extension	72^{o}C	5 min	1
Holding	4^{o}C	-	1

Note: The thermal cycling program listed above is optimized for primers with an annealing temperature at 55°C.

5. Analyze the amplification products by agarose gel electrophoresis.