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iScript™ IV TaqProbe One-Step RT-qPCR Kit

Catalog Number: D046-1, D046-2

Table 1. Kit Components and Storage

Kit Component	D046-1 (100 rxns)	D046-2 (200 rxns)	Storage	Stability
RT-qPCR 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-qPCR Master Mix (5X)	400 µL	800 µL		
ROX Reference Dye (25 µM)	100 µL	200 µL		
Low ROX Reference Dye (2.5 µM)	100 µL	200 µL		

Product Description

iScript™ IV TaqProbe One-Step RT-qPCR Kit is a complete qPCR system containing all necessary reagents for both reverse transcription and TaqMan Probe based qPCR amplification to occur in a single reaction qPCR reaction tube. RT-qPCR 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-qPCR Master Mix (5X) contains stabilizers and enhancers that optimize the two reactions in a real-time “single step”. This One-Step RT-qPCR Kit offers the end-users an efficient and reliable alternative to conventional “two-step” sequential RT-qPCR. Gene-specific primers must be used along with this kit.

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 qPCR tube on ice:

Component	Volume	Final Concentration
Total RNA or poly(A)$^+$RNA	x µL	2 pg-0.2 µg total RNA or 0.01 pg-5 ng mRNA
5× RT-qPCR Master Mix	4 µL	1×
10× RT-qPCR Enzyme Mix	2 µL	1×
ROX Reference Dye (25 µM) or Low ROX Reference Dye (2.5 µM) or No ROX	0.4 µL 0.4 µL -	500 nM 50 nM -
Forward Primer (10 µM)	0.6 µL	300 nM
Reverse Primer (10 µM)	0.6 µL	300 nM
TaqMan Probe	x µL	100-300 nM
Nuclease-free H_2O	to 20 µL	-

Note: 1. Gene specific primers must be used.
2. Amplicon should be <150 bp in size.
3. Check Table 1 for final concentration of ROX optimal for your instrument.

3. Mix 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 qPCR amplification.

Steps	Temperature	Duration	Cycle
cDNA Synthesis	50^{o}C	15 min	1
Initial Denaturation	95^{o}C	3 min	1
Denaturation	95^{o}C	15 sec	40
Annealing/Extension	60^{o}C	60 sec	
Melting Curve	According to the instrument guidelines		

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to RT-qPCR reaction.

Table 1. Recommended amounts of ROX Reference Dye for a specific instrument.

Instrument	Volume (20 µL rxn)	ROX Selection
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.4 µL	ROX Reference Dye (25 µM)
Applied Biosystems: 7500, 7500 Fast, Viia7. **Stratagene:** Mx3000P, Mx3005P, Mx4000	0.4 µL	Low ROX Reference Dye (2.5 µM)
BioRad: iCycler iQ, MyiQ, iQ5, CFX-96, CFX-384. **Eppendorf:** Mastercycler ep realplex. **Roche:** LightCycler 480, LightCycler 2.0. **Corbett:** Rotor-Gene 3000, 6000.	None	No ROX