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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 (20X)	100 µL	200 µL	-20°C in a non-frost-free freezer	The product is stable for one year when stored as directed.
RT-qPCR Master Mix (2X)	1 mL	2×1 mL		
RNase-free H_2O	1 mL	2×1 mL		
ROX Reference Dye (50 µM)	50 µL	50 µL		

Product Description

iScript™ IV TaqProbe One-Step RT-qPCR Kit is a complete system containing all necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube using gene-specific primers. RT-qPCR Enzyme Mix 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 contains stabilizers and enhancers that optimize the activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts.

The system enables highly sensitive detection from as few as 10 copies of a target gene, with a broad dynamic range that supports accurate quantification of high-copy mRNA from up to 1 µg of total RNA.

- **iScript IV Reverse Transcriptase** is a new version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme can synthesize cDNA at a temperature range of 42–60°C.
- **AccuStart Taq DNA polymerase** is recombinant Taq DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling, providing an automatic “hot start” in PCR for increased sensitivity, specificity, and yield.
- **RT-qPCR Master Mix** consists of a proprietary buffer system, $MgSO_4$, dNTPs, and stabilizers.

This one-step RT-qPCR kit has been formulated for use with fluorogenic primers or fluorogenic probe-based technology (e.g., TaqMan™ probes).

Applications

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

Recommendations and Guidelines for One-Step RT-qPCR

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, Eppendorf, ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, ABI Prism7000/7300/7700/7900, ABI Step One/Step One Plus. Optimal cycling conditions will vary with different instruments.

Template

Starting material can range from 1 pg to 1 µg of purified total RNA. If you are using purified mRNA, the amount of template may be as low as 0.5 pg. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with DNase I to remove any contaminating genomic DNA.

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 50 µM concentration. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per ml of RT-qPCR Master Mix
Applied Biosystems: ABI 7000, 7300, 7700, 7900HT, StepOne, StepOne Plus	20 µL
Applied Biosystems: 7500, 7500 Fast, Viia7, QuantStudio 3, QuantStudio 5, QuantStudio 6 Flex, QuantStudio 7 Flex. **Stratagene:** Mx3000P, Mx3005P, Mx4000	2 µL
BioRad: iCycler iQ, MyiQ, iQ5, CFX-96, CFX-384. **Eppendorf:** Mastercycler ep realplex. **Roche:** LightCycler 480, LightCycler 2.0.	None

Melting Curve Analysis

Melting curve analysis should always be performed during RT-qPCR to identify the presence of primer dimers and analyze the specificity of the reaction.

Reaction Setup and Conditions

Keep all components, reaction mixes and samples on ice.

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
2× RT-qPCR Master Mix	10 µL	1×
20× RT-qPCR Enzyme Mix	1 µL	-
RNA template	x µL	1 pg-1 µg
Forward Primer (10 µM)	0.5 µL	250 nM
Reverse Primer (10 µM)	0.5 µL	250 nM
Probe (10 µM)	0.5 µL	250 nM
Nuclease-free H_2O	to 20 µL	-

Note: 1. Gene-specific primers must be used.
2. Amplicon length should be approximately 70-150 bp.
3. Check the instrument to determine the ROX amount to be added to RT-qPCR Master Mix.

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	52°C	5 min	1
Initial Denaturation	95°C	10 sec	1
Denaturation	95°C	5 sec	40-45
Annealing/Extension	60°C	10-20 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.