

One-Step qRT-PCR Kit



Cat. No. SM307-1000

Size: 1000 Reactions

Cat. No. SM307-0100

Size: 100 Reactions

Store at -20°C

Description

The One-Step qRT-PCR Kit are compatible with TaqMan probes. The Kit provides a convenient, sensitive, and reproducible method to detect and quantify the RNA molecules by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The components for cDNA synthesis, PCR amplification, and quantification are combined in a kit, using gene-specific primers, probes, and target RNAs from either total RNA or mRNA. The kit consists of three major components: RT/ HotStar Taq Mix, 2X Reaction Mix, and 50 mM Magnesium Sulfate (MgSO₄). The RT/ HotStar Taq Mix contain a mixture of Reverse Transcriptase (RTase) and HotStar Taq DNA polymerase for optimal cDNA synthesis and PCR amplification. The RTase is modified from the Moloney Murine Leukemia Virus (M-MLV) RTase, engineered to reduce RNase H activity and increase thermal stability. The HotStar Taq DNA polymerase is Taq DNA polymerase complexes with a proprietary antibody that blocks activity at ambient temperatures. Activity is restored after the enzyme activation step at 95°C, thereby providing an automatic “hot-start” for Taq DNA polymerase in PCR, increasing the sensitivity and specificity of PCR reaction. The 2X Reaction Mix consists of 6 mM MgSO₄, deoxyribonucleotide triphosphates (dNTPs), and stabilizers provide a proprietary buffer system optimized for reverse transcription and PCR amplification. One addition tube of 50 mM MgSO₄ is included in the kit. For COVID-19 detection, the optimal concentration is 4 mM. An additional 0.5 µl for each reaction is recommended.

Feature

- Time efficiency – included most reagents for qRT-PCR reaction.

Applications

- Detection of expressed genes.
- Examination of transcript variants.

Kit Contents

Contents	SM307-1000	SM307-0100
RT/ HotStar Taq Mix	500 µl	50 µl
2X Reaction Mix	12.5 ml	1.25 ml
50 mM MgSO ₄	1 ml x 2 vials	200 µl

Quality Control

The quality of the One-Step qRT-PCR Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Reaction tubes and caps /qPCR plates and seals
- qPCR thermal cycling instrument
- Gene-specific primers and fluorogenic probe
- RNase inhibitor (optional)
- qPCR thermal cycling instrument
- DEPC- treat water
- ROX reference dye (optional)

Compatible Instruments

Depending on the use of fluorescent PCR instrument, check on the following table, optimal cycling conditions will vary with different instruments:

Product Name	qRT-PCR Thermal Cycling Instrument
One-Step qRT-PCR Kit	Applied Biosystems™ 7000, 7300, 7500, 7700, and 7900HT Real-Time PCR Systems Applied Biosystems™ GeneAmp™ 5700 Bio-Rad™ iCycler™ Agilent™ Mx3000P™, Mx3005P™, and Mx4000™ Corbett Research Rotor-Gene™ MJ Research DNA Engine Opticon™, Opticon™ 2, and Chromo4™ Real-Time Detector Cepheid SmartCycler™ thermal cyclers

One-Step qRT-PCR Kit Protocol

1. Thaw the One-Step qRT-PCR Kit, template RNA, primers, fluorogenic probe, ROX reference dye (optional) and DEPC-treat water on ice. Mix each solution well.
2. Set up the following reaction mixture, and reaction cocktails can be made when multiple reactions are being assembled.

Component	Volume (µl)	Final Concentration
2X Reaction Mix	12.5 µl	1X
Template RNA	Variable	1 pg-1 µg
Sense Primer (10 µM)	0.5 µl	200 nM
Anti-Sense Primer (10 µM)	0.5 µl	200 nM
50 mM MgSO ₄ (optional)	0.5 µl	
RT/ HotStar Taq Mix	0.5 µl	
Fluorogenic probe (10 µM)	0.25 µl	100 nM
ROX Reference Dye (optional)	Variable	
RNase Inhibitor (optional)	0.5 µl	20 U
Add DEPC treat water to	25 µl	

3. Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
4. Perform qRT-PCR reactions using the following cycling program:

Temperature (°C)	Standard Mode Process	Fast Mode Process	Cycles
50	15 minutes	5 minutes	1
95	2 minutes	2 minutes	1
95	15 seconds	3 seconds	40
60	30 seconds	30 seconds	

5. Collect the data and analyze results.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did qRT-PCR amplification with the kit.

Problem	Cause	Solution
No signal, or one or more signals detected late in qRT-PCR	Wrong cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Be sure that the cycling conditions include the initial step for cDNA synthesis, RTase Inactivation / Hot-start <i>Taq</i> Activation, and the specified times for denaturation and annealing/extension.
	Wrong or no detection step	Ensure that fluorescence detection takes place during the combined annealing/extension step.
	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the qRT-PCR using the new dilutions.

Related Ordering Information

Cat. No.	Description	Size
SN017-0100	Total RNA Isolation Kit (Blood/ Cultured Cell/ Fungus)	100 Reactions
SN020-0100	Total RNA Isolation Kit (Plant)	100 Reactions
SN021-0100	Total RNA Isolation Kit (Tissue)	100 Reactions
SR001-2500	RiboIN RNase Inhibitor, 40 U / μ l	2500 U

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.