One-Step RT-PCR Kit

Cat. No.: SM300-0100 Size: 100 Reactions
Store at -20°C

Description

The One-Step RT-PCR Kit provides a convenient, sensitive, and reproducible method to detect and analyze the RNA molecules by reverse transcription polymerase chain reaction (RT-PCR). The components for cDNA synthesis and PCR amplification are combined in a kit, using gene-specific primers and target RNAs from either total RNA or mRNA. Reverse transcription automatically follows PCR cycling without additional steps. The kit consists of three major components: RT/ HotStar Taq Mix, 2X Reaction Mix, and 50 mM Magnesium Sulfate ($MgSO_4$). 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 $MgSO_4$, deoxyribonucleotide triphosphates (dNTPs), and stabilizers provide a proprietary buffer system optimized for reverse transcription and PCR amplification. One addition tube of 50 mM $MgSO_4$ is included in the kit. The 2X Reaction Mix is well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the separate 50 mM $MgSO_4$ buffer to increase the magnesium concentration.

Features

- Time efficiency – included most reagents for reverse transcription and PCR reaction.

Kit Contents

Contents	SM300-0100
RT/ HotStar Taq Mix	50 µl
2X Reaction Mix	1.25 ml
50 mM $MgSO_4$	200 µl

Quality Control

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

Required Materials

- PCR tubes
- RNase-free H_2O
- PCR instrument
- DNA electrophoresis equipment

One-Step RT-PCR Kit Protocol

1. Thaw the One-Step RT-PCR Kit, template RNA, primers and RNase-free H_2O 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	5 pg-0.5 µg
Sense Primer (10 µM)	0.5 µl	200 nM
Anti-sense Primer (10 µM)	0.5 µl	200 nM
RT/ HotStar Taq Mix	0.5 µl	
Add RNase-free H_2O to	25 µl	

Note:

1. The cycling conditions may have to be optimized for different sequences. Annealing and extension steps are separated (three-step cycling).
2. For each primer is generally optimal, a final concentration of primers is 200 nM. However, for best results, a primer titration using 150-500 nM is recommended.
3. Efficient cDNA synthesis can be achieved in 15-30 minutes incubation at 42-60°C. It is recommended that 15 minutes incubation at 50°C be used as a general starting point.
4. For most targets up to 3 kb, 0.5 µl of RT/ HotStar *Taq* Mix is sufficient. Usage amount of RT/HotStar *Taq* Mix is proportional to template RNA.
5. Gene specific primers (GSP) are recommended. Use of oligo(dT) or random primers are not recommended as they result in generation of non-specific products in the cDNA synthesis and the amount of RT-PCR product may be reduced.

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

Process	Temperature (°C)	Time	Cycles
cDNA synthesis	45-55	15-30 minutes	1
RTase Inactivation / Hot-start Taq Activation	95	2 minutes	1
Denaturation	95	15 seconds	35-40
Annealing	55-60	30 seconds	
Extension	68-72	1 minute /kilo base	
Final Extension	72	5-10 minutes	1

Note: In Perkin-Elmer Model 480 cycler, the denaturation step, use 30 seconds instead of 15 seconds.

5. Analyze the amplification products by DNA electrophoresis.

Troubleshooting

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

Problem	Cause	Solution
Low yield of PCR products	Incomplete concentration of start materials	Use the appropriate method for the RNA preparation based on the amount of the starting materials.
	RNA degraded	Avoid repeated freeze / thaw cycles of the sample.

Problem	Cause	Solution
Low yield of PCR products	RNA degraded	Keep DNA preparations on ice or frozen in order to avoid the degradation.
	RNase contaminant	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, e.g.: RNase inhibitor.

Related Ordering Information

Cat. No.	Description	Size
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
BK001	BLooK LED Transilluminator	1 Set
SD010-R500	1 Kb DNA Ladder RTU	500 µl
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	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.