

ONE-STEP RT-PCR PreMix Kit

Cat. No. 25101 50 Rxn

DESCRIPTION

The ONE-STEP RT-PCR PreMix Kit is designed for easy, convenient and sensitive RT-PCR (cDNA synthesis and PCR) from RNA templates. Each tube of ONE-STEP RT-PCR PreMix Kit contains all components (required to synthesize your single stranded cDNA and its PCR reaction). Because the kit contains OptiScript™ RT System, you can perform highly efficient and specific reverse transcription reaction. OptiScript™ RT System designed for reverse transcription of any RNA quantity from 1pg to 4µg. And the kit contains *i-StarTaq*™ DNA polymerase, you can perform hot-start PCR procedure, eliminate extension from nonspecifically annealed primers and primer-dimers in the first cycle ensuring highly specific and efficient PCR. The kit contains also stabilizing buffer, the activity of contained enzymes (reverse transcriptase, *Taq* DNA polymerase) maintained for long time.

STORAGE

Store at -20 .

KIT CONTENTS

ONE-STEP RT-PCR PreMix 50 Rxn

Component in 20 µl reaction

OptiScript™ RT System
RT-PCR buffer (10 ×)
dNTPs
i-StarTaq™ DNA polymerase
Stabilizing buffer

CHARACTERISTICS

- The kit contains all the reagents required for the synthesis of cDNA and its amplification, you can perform easily RT-PCR reaction.
- OptiScript™ RT System are included in the iNtRON's ONE-STEP RT-PCR PreMix Kit and provide highly efficient and specific reverse transcription.
- *i-StarTaq*™ DNA polymerase included in the iNtRON's ONE-STEP RT-PCR PreMix Kit provides hot-start PCR for highly specific amplification.
- The kit also contains stabilizing buffer, the stability of contained enzymes maintained for a long time.

PROTOCOL

1. Dispense 8 µl of ONE-STEP RT-PCR PreMix Kit into PCR tubes.
2. Add RNA templates and gene specific primers into the upper PCR tubes.
Note : Use the same amounts of gene specific primers as usual PCR reaction or two fold reverse primer recommended.
3. Add distilled water into the tubes to a total volume of 20 µl.
4. Mix the mixture thoroughly.
5. (Option) Add mineral oil.
Note : This step is unnecessary when using a thermal cycler that employs a top heating method (general methods)
6. Perform RT-PCR reaction of samples as following process using PCR machine.

ONE CYCLE	
Reverse transcription reaction	45 / 30min
Denaturation of RNA : cDNA hybrid	94 / 5min
3-STEP CYCLING	
Denaturation	94 / 20-60sec.
Annealing	45-68 / 20-60sec.
Extension	72 / 1min/kb
Number of cycles : 25-40	
ONE CYCLE	
Final Extension	72 / 5min

REACTION COMPONENTS FOR RT-PCR

Components	Volume / reaction	Final concentration
ONE-STEP RT-PCR PreMix Kit	8.0 µl / tube	—
Template RNA	Variable	
Forward primer	Variable	0.5 pM
Reverse primer	Variable	0.5 pM
RNase inhibitor(optional)	Variable	5-10 units/reaction
RNase-free water	Variable	
Total volume	20.0 µl	

* Use the same amount of reverse primer or two fold reverse primer.