One Step RT-PCR Kit

Store at -20℃

Description

The Omega Bio-Tek's One Step RT-PCR kit is designed for the sensitive, reproducible, end-point detection and analysis of RNA molecules by RT-PCR. Using this kit you can perform both cDNA synthesis and PCR amplification in a single tube using gene-specific primers and target RNAs from either total RNA or mRNA. The system uses an mixture of M-MLV Rtase (RNase H-), Taq DNA polymerase and RNase Inhibitor Mix in an optimized reaction buffer, and can detect a wide range of RNA targets. The amount of starting material can range from 10ng to 1μg of total RNA.

Product features

Using this kit you can perform both cDNA synthesis and PCR amplification in a single tube using gene-specific primers and target RNAs from either total RNA or mRNA.

The Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability.

aq DNA Polymerase is a high quality DNA polymerase. Used in this kit can obtain sensitivity, specificity and yield product.

The 5× reaction buffer included in the kit consists of a proprietary buffer system that has been optimized for reverse transcription and PCR, Mg^{2+}, stabilizers, dNTPs. Sufficient reagents are provided for 25 or 100 reactions of 50 μl each.

Kit components

Cat.No.	TQ2601-01	TQ2601-02
Preps	25 preps	100 preps
5 x Reaction Buffer (with dNTPs, 10mM)	250 μl	1 ml
M-MLV RTase / Taq DNA Polymerase Mix	25 μl	100 μl
Nuclease-Free Water	2×1 ml	8×1 ml

Quality Control

To test for RNase, DNase and Endonuclease activity found there is no contaminant .The product is tested functionally 100ng and 1μg of chicken liver total RNA as the template for amplification of a 1.5kb target(40cycles).

Important Guidelines

RNA

- Hight quality intact RNA is essential for successful full-length cDNA synthesis.
- For low copy-number genes or longer target , use more starting material(>20ng total RNA).
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained.
- We recommend the E.Z.N.A.® total RNA kit or RNA-Solv® Reagent for isolation of total RNA.

Primers

- In this kit, primers should use gene specific primers(GSPs) rather than Oligo(dT) or random primers.
- A final primer concentration of 0.2 μM for each primer is generally optimal.
- Design primer that anneal to the mRNA sequence in exons on both sides of an intron or exon/exon boundary, to allow differentiation between the amplified cDNA and potential contaminating genomic DNA.
- Primers should not be self-complementary or complementary to each other at the 3' ends.

Reactions

- You can preheated the thermal cycler to 40℃ before setting up the reaction.
- Keep all components, reaction buffer and samples on ice. After preparation of the reaction, transfer them to the preheated thermal cycler and immediately start the RT-PCR program.
- Efficient cDNA synthesis can be accomplished in a 15-30 min incubation at 37-42℃
- For long targets , more M-MLV RTase / Taq DNA Polymerase Mix is sufficient.

Protocol

1. **Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification, as follows :**

cDNA sythesis	1 Cycle	37-42℃ for 15-30 min
Denaturation	1 Cycle	94℃ for 5 min
PCR amplification	35-40 Cycle	94℃ for 30 s 50-60℃ for 30 s 72℃ for 1 min/kb
Final extension	1 Cycle	72℃ for 5 min
End	Hold	4℃

2. Add the following to a 0.2 ml thin-walled PCR tube (nuclease-free). For multiple reactions, you can prepare a master mix to minimize reagent loss and enable accurate pipetting.

Component	Volume
5×Reaction Buffer	10μl
Template RNA(10 ng-1 μg)	xμl
Sense primer(10 μM)	1μl
Anti-sense primer(10 μM)	1μl
M-MLV RTase / Taq DNA Polymerase Mix	1μl
Nuclease-free water	Up to 50μl

Note: you can verify absence of genomic DNA in RNA preparations by omitting the M-MLV RTase / omega Taq DNA Polymerase Mix and substituting 2.5 units of Taq DNA Polymerase in the reaction.

3. **Gently mix and make sure that all the components are at the bottom of the amplification tube.** Centrifuge briefly if needed. Depending on the thermalcycler used, overlay with silicone oil if necessary.

4. **Place the reaction in the preheated themal cycler programmed as described above.** Collect the data and analyze the results.

The above cycling conditions were established and tested using a MJ Rearch PTC-200 .You may need to adjust these conditions for other thermal cyclers.

Ordering Information

Cat.No.	TQ2601-01	TQ2601-02
Omega one step RT-PCR kit	25 reaction	100 reaction