Ver.24081

OneStep RTPCR Kit

Cat.No. ODR61

Description: The OneStep RTPCR Kit contains all necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube. Components for both cDNA synthesis and PCR are combined in a single tube, using gene-specific primers and target RNAs from either total RNA or mRNA. Reverse transcription automatically follows PCR cycling without additional steps. The OneStep RTPCR Kit consists of two major components: Enzyme Mix and 5X Reaction Mix, provided a convenient format for highly sensitive and specific RT-PCR using any RNA template. The Enzyme Mix is for optimal cDNA synthesis and PCR amplification. The enzyme mix uses a mixture of M-MLV Reverse Transcriptase and HotStart *Taq* DNA polymerase in an optimized reaction buffer. The 5X Reaction Mix consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, Mg^{2+} optimized for universal use, deoxyribonucleotide triphosphates, and stabilizers.

Storage

- Store at –20°C for 12 months
- Prepare aliquots to avoid multiple freeze-thaw cycles.

Features

Component	Volume
Enzyme Mix	100µL
5X Reaction Mix	550µL

Recommended PCR mixture and cycling

PCR mixture (Reaction vol. 50µL)		Cycle		
5X Reaction Mix	10µL	45-55°C	15-30 min	×1
Forward primer (10pmol/µL)	1µL	94°C	5 min	×1
Reverse primer (10pmol/µL)	1µL	94°C	15 sec	×35-40
Template RNA (1pg - 1µg)	-µL	55-60°C	30 sec	
Enzyme Mix	2µL	68-72°C	1 min/kb	
Add D.W to	50µL	72°C	5-10 min	×1

Note: You may modify the amount of template, extension time, annealing temperature, and the number of PCR cycle according to the target size, primers Tm and the type of templates for amplification.

Important Parameters

1. RNA

• High quality intact RNA is essential for successful full-length cDNA synthesis.

• RNA should be avoiding of any RNase contamination and aseptic conditions should be maintained.

2. Primers

• 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 one-step procedure and the amount of RT-PCR product may be reduced.

• A final primer concentration of 0.2µM for each primer is generally optimal. For best results, a primer titration using 0.15-0.5µM is recommended. Primers should not be self-complementary or complementary to each other at the 3' ends.

3. Magnesium Concentration

• The magnesium is included in the 5X Reaction Mix at a final concentration of 2.3mM, which works well for most RT-PCR.

4. dNTPs Concentration

• dNTPs are included in the 5X Reaction Mix, which is optimal for most reactions.