Catalog No. RT0201-02

Introduction

One-Step RT-PCR is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA. This system uses a mixture of MMLV Reverse Transcriptase and Hot start *Taq* DNA polymerase in an optimized reaction buffer, and can detect RNA targets up to 4.0 kb. The amount of input total RNA can range from 0.01 pg to 2 µg. Sufficient reagents are provided for 100 amplification reactions of 50 µl each.

Kit Contents

Contents	Volume
Enzyme mix: MMLV RT/ Hot start *Taq* Mix	50 µl
2× Reaction mix (optimized buffer contains dNTP and Mg)	1.25 ml
5 mM Magnesium Sulfate	1.0 ml

Storage

Store at -20 ℃, One-Step RT-PCR is stable for 2 year when stored at -20 ℃.

Features

• Convenient one-tube setup
• Optimized RT-PCR buffer and robustness
• High sensitivity, specificity, and reproducibility

Recommended RT-PCR reaction assembly

The following protocol is suggested as a starting point.

Components	**50 ul Rxn**	**Final Concentration**
2× Reaction mix	25 ul	1×
Enzyme mix	1.0 ul	
Forward Primer (10 uM)	1.0 ul	200 nM
Reverse Primer (10 uM)	1.0 ul	200 nM
RNA template	× ul	Variable (0.01pg - 2ug)
Final Volume (ul)	50 ul	

* If needed, the magnesium concentration can further be optimized

1. Assemble the reaction on ice.
2. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification.

cDNA synthesis: 1 cycle: 40–50 ℃ for 10 min

Denaturation: 1 cycle: 94 ℃ for 2 min

PCR amplification: 40 cycles:

94 ℃ for 15 s

55-65 ℃ for 30 s

68 ℃ for 1 min/kb

3. Analyze RT-PCR amplified products by gel electrophoresis.