LaboPass™ One-Step RT-PCR kit

Cat. No.
CMRO050 / CMRO100

COSMO GENETECH

Lot No.

Contents

Cat. No	CMRO050	CMRO100
Enzyme Mix	50 reactions	100 reactions
2X Reaction Mix	1.5 ml	1.5 ml x 2 vials
Nuclease-free water	1.5 ml	1.5 ml x 2 vials

Description

The LaboPass™ One-Step RT-PCR kit offers a convenient system to perform both cDNA synthesis and PCR amplification with genespecific primers and RNA templates in a single tube. The system consists of two major components : Enzyme mix and 2X Reaction Mix. Enzyme mix is a blend of Labopass™ M-MuLV Reverse transcriptase, IP-*taq* polymerase and RNase inhibitor. 2X Reaction Mix is formulated to enable both reverse transcription and PCR amplification efficiently. Therefore, this kit will offer a rapid and easy method to detect a broad size range of RNA targets with high sensitivity.

Applications

- Qualitative or quantitative analysis of gene expression
- Detection or quantification of RNA viruses
- cDNA amplification for gene cloning

Enzyme Mix

Enzyme Mix includes M-MuLV Reverse transcriptase, which is highly sensitive and can generate long cDNA strands. This mix also contains RNase Inhibitor to protect RNA templates from degradation. IP-*taq* for PCR amplification is high efficiency *Taq* polymerase. This enzyme possesses 5` to 3` exonuclease activity, but lacks a 3` to 5` exonuclease proofreading activity.

2X Reaction Mix

2X Reaction Mix has been optimized to allow both reverse transcription and PCR amplification to occur in the same reaction across a wide range of templates.

Storage and Stability

Store at -20°C until ready for use. LaboPass™ One-Step RT-PCR kit is stable for 12 months. Avoid repeated freeze thawing.

Quality Control

Nuclease activity test

0.5 μg of supercoiled pUC19, λ DNA, and λ DNA/HindIII DNA are incubated with 1 μl of Enzyme Mix in 1X Reaction Mix for 10 hours at 37°C and 72°C. Following incubation, nicking, cutting or smearing of the DNA is not detected on an agarose gels.

The protein purity

As judged by Coomassie blue staining of SDS-PAGE, the enzyme purity is more than 95%.

Standard reaction protocol

1. Mix the following reaction components in a sterile PCR tube and briefly spin-down

2X Reaction Mix	25 μl
10 μM Forward primer	1 μl
10 μM Reverse primer	1 μl
Enzyme Mix	2.5 μl
RNA template	100 pg to 1 μg according to target abundance
Nuclease-free wate	Up to 50 μl

2. Cycling condition

cDNA synthesis
1 cycle : 50°C for 30 ~ 60 minutes

RT inactivation / Denaturation
1 cycle : 95°C for 5 minutes

PCR amplification
30 ~ 40 cycle : 95°C for 15 ~ 30 seconds
50 ~ 65°C for 30 seconds
(annealing temperature has to be optimized empirically)
72°C for 1 minutes per kbp

Final extension (optional)
1 cycle : 72°C for 5 minutes

For laboratory use only

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