

OneStep RT-PCR Premix

Product:	OneStep RT-PCR Premix
CATALOG#:	BN0020-0500
Concentration:	2X
Volume:	500ul X2
Storage:	-20°C

Description

The OneStep RT-PCR Premix is a ready-to-use, 2X concentrated premix that contains all the reagents (except primers and template) needed for running RT-PCR in a single tube. Conventional RT-PCR kits employ a two step RT-PCR, which is performed sequential reactions and separately prepared reactions mixtures. These additional steps are undesirable, as they increase the risk of contamination.

One step RT-PCR premix minimizes the risk of contamination and increases the sensitivity of RNA amplification.

Content

2X One step RT-PCR Premix containing:

- Hotstart Taq DNA polymerase.
- Reverse transcriptases.
- dNTP mix including dATP \ dCTP \ dGTP \ dTTP
- 5 mM MgCl₂

OneStep RT-PCR Premix performance test

High sensitivity of RNA amplification by one step RT-PCR is confirmed by using 10 copies viral RNA as the template(amplified fragment: 0.5kb)

Procedure

A. Preparation of the RT- PCR master Mix

1. Preparing a master mix as follows:

Component	Volume / reaction	Final conc.
2x One-step RT-PCR Premix	12.5 µl	1X
Forward Primers (10µM)	variable	0. 6~1.0 µM
Reversed Primers (10µM)	variable	0. 6~1.0 µM
H ₂ O	upto 23.0 µl	

Mix the master mix thoroughly by pipetting up and down.

2. dispense 23µl volumes into PCR tubes .

3. Added 2µl of the RNA . Mix carefully by pipetting up and down.

B. Performing One Step RT- PCR

1. Program your instrument as follows:

Reverse transcription :	30 min	50~60°C
Initial PCR activation step :	10 min	95°C
3-step cycling Denaturation :	0.5 min	94°C
Annealing :	0.5 min	50~68°C
Extension :	1 min	72°C

2. PCR tubes are still on ice. Wait until the thermal cycle has reached 50°C. Then place the PCR tubes in the thermal cycle and start the RT-PCR program

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