USER GUIDE

ARROW-Script The One™ RT-PCR Mix (2X)

Cat. ARPMX02050
Qty. 50 r / 1.25 ml

Content and Storage

ARROW-Script The One™ RT-PCR Mix (2X)
Cat. ARPMX02050
Store at -20 ℃

Contents

Cat.ARPMX02050

ARROW-Script The One™ RT-PCR Mix (2X) ..... ***50r / 1.25 ml***

Content :
Hotstart Taq DNA polymerase.
Reverse transcriptase
dNTP mix including dATP、dCTP、dGTP、dTTP
5mM MgCl2

Introduction

ARROW-Script The One™ RT-PCR Mix (2X) 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.

ARROW-Script The One™ RT-PCR Mix (2X) minimizes the risk of contamination and increases the sensitivity of RNA amplification.

ARROW-Script The One™ RT-PCR Mix (2X) 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)

ARROWTEC

Standard Protocol

Set up each reaction as follows :

component	Vol./reaction
ARROW-Script The One™ RT-PCR Mix (2X)	25 µl
5-50 µM forward primer	1 µl
5-50µM reverse primer	1 µl
* template DNA	10 pg - 1 µg
DNase Free water to **50 µl**	

Program your instrument as follows :

Step	Time	Temp
Reverse transcription	30 min	50~60 ℃
Initial PCR activation step	10 min	95 ℃
3-step cycling Denaturation	0.5 min	94 ℃
Annealing	0.5 min	50~68 ℃
Extension	1 min	72 ℃
Number of cycle : 35 - 40 cycles		

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

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

Troubleshooting

Problem	Possible cause	Possible solution
No amplification product	No cDNA synthesis (temperature too high)	For the cDNA synthesis step, incubate <55˚C.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	Not enough starting template RNA	Increase the concentration of template RNA; use 100 ng to 1 µg of total RNA.
	RNA has been damaged or degraded	Replace RNA if necessary.
	Annealing temperature is too high	Decrease temperature as necessary.
	Extension time is too short	Set extension time for at least 60 seconds per kb of target length.
Unexpected bands after electrophoretic analysis	Nonspecific annealing of primers	Vary the annealing temperature, Optimize the magnesium concentration for each template and primer combination.
	Primers formed dimers	Design primers without complementary sequences at the 3˙ ends.