USER GUIDE

ARROW-Script RT Mix (2X)

Cat. ARPMX01050, ARPMX01050T, ARPMX01050R
Qty. 500 µl

Content and Storage

ARROW-Script RT Mix (2X)
Cat. ARPMX01050, ARPMX01050T, ARPMX01050R

Store at -20 ℃

Contents
Cat. ARPMX01050
DNase / RNase Free Water ..... 500 µl
ARROW-Script RT Mix(2X) ..... 500 µl
component :
Reverse transcriptase, 2X reaction buffer, dNTP mix
oligo d(T) primers and random hexamers

Contents
Cat. ARPMX01050T
DNase / RNase Free Water ..... 500 µl
ARROW-Script RT Mix(2X) ..... 500 µl
component :
Reverse transcriptase, 2X reaction buffer, dNTP mix
oligo d(T) primers

Contents
Cat. ARPMX01050R
DNase / RNase Free Water ..... 500 µl
ARROW-Script RT Mix(2X) ..... 500 µl
component :
Reverse transcriptase, 2X reaction buffer, dNTP mix
random hexamers

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Introduction

ARROW-Script RT Mix (2X) provides a sensitive and easy-to-use solution for two-step RT-PCR. This Mixture includes just one tube — comprehensive of reagents required for successful reverse transcription. Mixture containing reverse transcriptase(M-MLV), dNTP mixture, reaction buffer and oligo d(T) or Random hexamers or Mixture of oligo d(T) and Random hexamers. The unique blend of oligo (dT) and random hexamer primers in the reaction mix works exceptionally well with a wide variety of targets.

ARROW-Script RT Mix (2X) produces excellent results in both real time and conventional RT-PCR.

Standard Protocol

Incubate the template RNA 0.1-5 µg in RNase free water to final volume 10 µl and stand at 65℃ for 15 min, and then keep on ice.

Set up each reaction as follows:

component	Vol./reaction	Final Conc.
ARROW-Script RT Mix(2X)	10 µl	1X
RNase Inhibitor*	1 µl (40 units/µl)	
DNase/RNase Free Water	Variable	---------
Template RNA	Variable	Variable
TOTAL Volume	20 µl	---------

* RNase Inhibitor (Cat. RB0478 ,2,000units, 40 units/µl). When using less than 50 ng of starting RNA, the addition of RNase Inhibitor is essential.

Standard Protocol

Perform the reaction under the following condition 42℃ for 30-60 minutes.

The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 50℃) for the highly structured RNA when treated 37-42℃ for 10mins later.

Heat at 70℃ for 15 minutes.(Inactivate the reaction)

NOTE :
The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 2 units of RNase H(Cat. GR0610, 10 units/ µl) and incubate at 37℃ for 20 min.*

PCR Protocol

Use only 10-15% (2-3µl) of the first-strand reaction for PCR.
Higher volumes may not increase amplification and may result in decreased amounts of PCR product.

Add the following components to a PCR tube .
2.5 µl ..... 10X PCR Buffer
0.5 µl ..... 10 mM dNTPs Mixture(10 mM each dATP, dGTP, dCTP and dTTP)
0.5 µl ..... 10µM Forward primer
0.5 µl ..... 10µM Reverse primer
2 units..... Taq DNA polymerase
2 µl ..... cDNA for First-Strand reaction
Sterile , DNase Free water to **25µl**