WizScript™ RT Master

• W2203 **100 rxn**

Description

WizScript™ RT Master is a complete system for the efficient synthesis of first strand cDNA from RNA templates.
WizScript™ RT Master inclueded MMLV RTase (RNase H-) which is an RNA-dependent DNA polymerase that is used in cDNA synthesis with long RNA templates. The lack of RNase H activity is important in this application in that RNase H activity will start to degrade template during long incubation times which are required for producing long cDNAs. RNase H minus RT enables preparation of long cDNAs and libraries containing a high percentage of full-length cDNA.
The kit is also supplied with both oligo dT and random primers. The oligo dT anneals selectively on the poly(A) tail of mRNA. Random primers do not require the presence of poly(A). Therefore, they can be used for transcription of the 5'-end regions of mRNA. Gene-specific primers may also be used with the kit. The first strand of cDNA can be directly used as a template in PCR.

Kit Contents

Contents	W2203
WizScript™ RT Master	1 ml
Oligo dT20 (50 pM)	100 µl
Random Hexamer (50 pM)	200 µl
RNase-Free Water	1 ml

• RT Master mix containing WizScript™ RTase, RNase inhibitor, stabilizer and reaction buffer containing optimized concentrations of MgCl2, dNTPs and DTT.

Storage Conditions

Store all components at -20°C in a non-frost-free freezer.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Quality Authorized by : Jamie Ahn 

Standard Protocol

1. Prepare the following mixture in a microtube.

Component	Volume
RT Master mix	10 µl
Oligo dT (Random hexamer)	1 µl (2 µl)
Template RNA*	< 5 µg
RNase free water	up to 20 µl

* Notes : Recommended amounts of RNA template and primers for first-strand cDNA synthesis.
(1) RNAs : total RNA : 10 ng ~ 5 µg
poly(A)+ RNA : 1 ng ~ 500 ng

(2) Primers : Oligo dT20 : 50 pM
Random hexamer : 50 ~100 pM
Gene-Specific Primer : 15 ~ 20 pM

2. Place tube in a thermal cycler programmed as follows:
25°C / 10 min.
42°C / 30 min.
85°C / 5 min.
4°C / Hold

3. Synthesized cDNA is immediately used as template for PCR or store at -20°C. The cDNA can be diluted with TE buffer and stored at -20°C.

Notes

1. Isolation of poly(A)+ RNA from total RNA is not mandatory, however, doing so may improve the yield and purity of the final product.
2. RNA sample must be free of contaminating genomic DNA.
3. Unlike the oligo dT priming, which usually requires no optimization, the ratio of a random primer to RNA is critical in terms of the average length of cDNA synthesized in the reaction. Increasing the ratio of random primer/RNA will result in higher yield of shorter (~500 bp) cDNA, whereas decreasing this ratio will produce longer products.
4. The synthesized cDNA should be stored at -20°C.

RUO Research Use Only

wizbiosolutions THE INNOVATION OF GENOMICS
TEL : +82 (0)70 7605 5066 sales@wizbiosolution.com
FAX : +82 (0)31 624 3066 www.wizbiosolution.com