WizScript™ RT FDmix (Hexamer)

(Aliquot & lyophilized in 8-strip tube)

• W2205 **96 rxn**

Description

WizScript™ RT FDmix (Hexamer) is a complete system for the efficient synthesis of first strand cDNA from RNA templates and combines all the reagents necessary for successful cDNA synthesis in a convenient individually aliquot and lyophilized in single-tube, one-step format. The FDmix formulation saves time and reduces potential contaminating errors by eliminating several pipetting steps.

WizScript™ RT FDmix (Hexamer) inclueded WizScript™ RTase 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. And enhanced buffer allows for RT reaction temperatures up to 50°C. This can improve detection of more difficult targets as higher RT temperatures reduce nonspecific priming and facilitate melting of RNA secondary structures.

The kit is also inclueded random hexamer primers that consist of a mixture of oligonucleotides representing all possible hexamer sequences. During cDNA generation, random priming gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Random priming is incapable of distinguishing between mRNA and other RNA species present in the reaction. The first strand of cDNA can be directly used as a template in PCR.

Kit Contents

Contents	W2205
WizScript™ RT FDmix (Hexamer)	96 tubes

Applications

- First strand cDNA synthesis
- Full lenth cDNA library
- Real-time PCR
- PCR
- DNA labeling

Storage Conditions

Upon receipt, store all components at –20°C.

Note

Do not contaminate the WizScript™ RT FDmix (Hexamer) with template RNA used in individual reactions.

Quality Control

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

Quality Authorized by : Jamie Ahn 

Protocol

This standard protocol applies to a single reaction where only template, and water need to be added to the RT FDmix (Hexamer) tube.

1. Place the RT FDmix (Hexamer) tube on PCR tube rack.
2. Add the reaction component to the RT FDmix (Hexamer) tube as following table shows recommended component volumes:

Component	Volume
RT FDmix (Hexamer)	1 tube
Template RNA*	< 5 µg
RNase free water	up to 20 µl

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

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

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