Biozym cDNA Synthesis Kit

Product information

Up on arrival the kit should be stored at -20°C.

Component	331470X 10 rxn	331470L 125 rxn
Reverse Transcriptase	1 x 10 µl	1 x 125 µl
5× cDNA Synthesis Buffer	1 x 100 µl	1 x 500 µl
dNTP Mix (10 mM each)	1 x 20 µl	1 x 250 µl
RNase Inhibitor (40 U/µl)	1 x 5 µl	1 x 62.5 µl
Hexamer Primer (25 µM)	1 x 10 µl	1 x 125 µl
Oligo (dT) Primer (10 µM)	1 x 5 µl	1 x 62.55 µl
PCR Grade Water, ultrapure	1 x 1.5 ml	5 x 1.5 ml

For research use only.

1. Introduction

Biozym cDNA synthesis Kit provides all necessary components for cDNA synthesis for two-step quantitative reverse transcription PCR (qRT-PCR) applications. Total RNA, mRNA, viral RNA or in vitro transcribed RNA can be used as a template for reverse transcription.

The RNase Inhibitor serves as a potent non-competitive inhibitor of RNases. The combination of effective RNase inhibition, highly efficient cDNA synthesis and pure dNTPs allows high yields of cDNAs of more than 19 kb. The kit includes both random primers and oligo(dT) primers. Either of these or alternatively use of gene-specific primers can be choosen.

2. Notes

- The MMuLV RNase used shows RNase H activity (MMuLV RNase H+) and provides high sensitivity. RNase H activity in the RT enzyme facilitates annealing of PCR primers to the cDNA by degrading the RNA in the RNA-cDNA hybrid before PCR steps.
- Kit includes random primers and oligo(dT) primers
- Highly efficient synthesis of long cDNAs (≥ 19 kb)
- Temperatures up to 55°C

3. Technical support

For technical support please contact support@biozym.com

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4. Reaction Setup and Protocol

4.1. Prevention of cDNA synthesis reaction contamination

RNase contamination is an exceptional concern when working with RNA. RNase A, providing most threat to RNA integrity, is a highly stable contaminant of any laboratory. To prevent RNA from degradation and to minimize possibility of contamination during cDNA synthesis follow the guidelines below:

- Use separate clean areas for preparation of the samples and the reaction mixture.
- Use certified nuclease-free labware with aerosol filters.
- Wear fresh gloves when handling RNA and all reagents.
- Always analyze the integrity of RNA prior to cDNA synthesis.
- Use RNase free water and other reagents.
- To prevent RNA from degradation, add Ribonuclease inhibitor (optional) to the cDNA synthesis reaction (20 units for 20 µl reaction).

4.2. Reaction setup

- Thaw on ice and mix very well all reagents.
- Assemble and keep all reactions on ice.
- To use time and reagents effectively, always prepare master mix for multiple reactions. For a master mix volume, always calculate the number of reactions you need plus one additional.
- Combine the following in a RNase-free reaction tube:

Table 1. Pipetting instructions

Component	Volume	Final concentration
dNTP Mix (10 mM each)	2 µl	1 mM (each dNTP)
RNase Inhibitor, 40 U/µl (optional)	0.5 µl	1 U/µl
Oligo (dT)$_{12–18}$ (10 µM) – or *Hexamer Primer (25 µM)* – or *Gene Specific Primer (10 µM)*	*0.5 µl* *1 µl* *0.5 µl*	*0.25 µM* *1.25 µM* *0.25 µM*
5× cDNA Synthesis Buffer	4 µl	1×
RNA Template *total RNA – or* *mRNA (poly(A))*	 *0.1–1 µg* *50–500 ng*	
Reverse Transcriptase	1 µl	10 U/µl
PCR Grade Water	Variable	
Total volume	20 µl	

- Mix and spin down briefly.
- When using
  - Hexamer Primer, incubate 10 minutes at 30°C followed by 50–55°C for 20–60 minutes
  - Oligo (dT) or gene-specific Primer incubate at 50–55°C for 20–60 minutes.
- Inactivate enzyme at 99°C for 5 minutes.
- Spin down briefly.
- Store products at –20°C or proceed to next step, like PCR or qPCR.
- *Use maximum 10 µl of the cDNA synthesis reaction mix for PCR in 50 µl volume.*