Biozym cDNA Synthesis Kit

Product information

Store all kit components at -20°C with minimal exposure to light.
The kit may be stored at 4°C for short term use (up to 1 month). Multiple freeze/thaw cycles (up to 30) are possible with no loss of activity.

Component	331475S 25 rxn	331475L 125 rxn
20x MMuLV RTase (RNase Inhibitor included)	1 x 25 µl	5 x 25 µl
5× cDNA Synthesis Mix	1 x 100 µl	5 x 100 µl

For research use only.

1. Introduction

Biozym cDNA synthesis Kit provides in two tubes all necessary components for cDNA synthesis for quantitative reverse transcription PCR (qRT-PCR) applications with accurate and linear transcript representation. 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. The kit includes a ready to use combination of random hexamer and anchored oligo(dT) primers.

2. Notes

- The modified MMuLV Reverse Transkriptase is efficiently working even with high degree secondary structure templates.
- Incubation is recommended at 42°C for 30 minutes. If the RNA template contains high (>65%) GC regions potentially forming secondary structures an incubation temperature up to 55°C could be used.
- The cDNA Synthesis Mix includes random hexamer and oligo(dT) primers, 5 mM dNTPs, 15 mM $MgCl_2$ and enhancers with optimized concentration for qPCR dedicated cDNA synthesis.
- The kit is recommended to be used with 4.0 pg to 0.4 µg of total RNA or oligo(dT) purified mRNA. For increased yield up to 5 µg of total RNA may be added.

3. Technical support

For technical support please contact support@biozym.com

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4. Reaction Setup and Protocol

- Thaw the 5x cDNA Synthesis Mix (on ice) and mix very well.
- Assemble and keep all reactions on ice.
- Prepare a 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 (20 µl total rxn volume)	Final concentration / Notes
5x cDNA Synthesis Mix	4.0 µl	1x, see 5.2.
20x RTase	1.0 µl	1x, add before total RNA as RTase is blended with RNase inhibitor
RNA template *total RNA or mRNA (poly(A))*	X µl	4.0 pg – 0.4µg, see 5.3.
PCR Grade Water	X µl variable, up to 20 µl	

- Mix and spin down briefly.
- Incubate 30 minutes at 42°C (see 5.1.)
- Inactivate enzyme 10 minutes at 85°C.
- Store products at –20°C or proceed to next step, like PCR or qPCR.
- Use maximum 2.0 – 4.0 µl of the cDNA synthesis reaction mix for PCR in 20 µl volume. If needed the created cDNA could be diluted with PCR Grade Water to extend the volume

5. Notes about components and reaction conditions

5.1. Incubation

Incubation is recommended at 42°C for 30 minutes. If the RNA template contains high (>65%) GC regions potentially forming secondary structures an incubation temperature up to 55°C could be used.

5.2. Biozym cDNA Synthesis Mix

The cDNA Synthesis Mix includes random hexamer and anchored oligo(dT) primers, 5 mM dNTPs, 15 mM $MgCl_2$ and enhancers.

5.3. RNA template

The kit is recommended to be used with 4.0 pg to 0.4 µg of total RNA or oligo(dT) purified mRNA. For increased yield up to 5 µg of total RNA may be added (linear and complete cDNA synthesis isn't guaranteed with this high RNA amount in every case).