Biomiga Inc.
The Inventor of EZgene™ and ViraTrap™ Systems

Express 1st Strand cDNA Synthesis System

Catalog No. FSE01-01: Biomiga Express 1st Strand cDNA Synthesis System, Size: 20 reactions
Catalog No. FSE01-02 Biomiga Express 1st Strand cDNA Synthesis System, Size: 50 reactions
Catalog No. FSE01-03: Biomiga Express 1st Strand cDNA Synthesis System, Size: 100 reactions

Store at -20°C
Biomiga Express 1st Strand cDNA Synthesis System is stable for 2 years when stored at -20°C (non-frost-free).

Description
Biomiga Express 1st Strand cDNA Synthesis System is optimized to synthesize high yield cDNA from total or poly(A)$^+$ purified RNA. The system can be used with a broad range of RNA template concentrations, from as low as 10 pg to as high as to 5 µg of total RNA. Biomiga Express 1st Strand cDNA Synthesis reverse transcriptase is a mixture of reverse transcriptase, an engineered MMLV RT and a ribonuclease inhibitor protein. A wide range of RNA targets from 100 bp to 10 kb can be detected with this system. It is ideally suited for gene isolation, high throughput expression profile studies using real-time quantitation, and end-point RT-PCR. The system contains all the components needed for first-strand cDNA synthesis. The reagents are sufficient for 50 or 200 cDNA synthesis reactions of 20 µl reaction volume.

Components

Components	2 Rxn	(20 Rxn)	(50 Rxn)	(200 Rxn)
• RT and RNase inhibitor mix	3 µl	20 µl	50 µl	200 µl
• cDNA Synthesis Buffer	30 µl	250 µl	500 µl	2.0 ml
• RNA annealing buffer OT (contains Oligo $(dT)_{20}$)	15 µl	125 µl	250 µl	1.0 ml
• RNA annealing buffer RH (contains Random Hexamer)	15 µl	125 µl	250 µl	1.0 ml
• Nuclease-free water	50 µl	1.0 ml	1.5 ml	2.0 ml

Product Qualification
Biomiga 1st Strand cDNA Synthesis System is functionally tested for amplification of a 1.1-kb RNA target from 10 ng total RNA.

1st Strand cDNA Synthesis Protocol

1. Prepare RNA/Primer mixtures.
   (a). Using Oligo $(dT)_{20}$

Components	1X Rxn
RNA (10pg-5 µg)	x µl
RNA annealing buffer OT	5.0 µl
Nuclease-free water	to 10 µl

(b). Using Random Hexamer

Components	1X Rxn
RNA (10pg-5 µg)	x µl
RNA annealing buffer RH	5.0 µl
Nuclease-free water	to 10 µl

2. Incubate the above sample at 65 °C for 3 minutes and place on ice until use.

3. Prepare the following RT reaction mixture on ice.

Components	1X Rxn	10X Rxn
cDNA Synthesis Buffer	9.0 µl	90 µl
RT and RNase inhibitor Mix	1.0 µl	10 µl
Total	10 µl	100 µl

4. Add 10 µl of reaction mixture to each RNA/Primer mix, mix gently, and then centrifuge 3 seconds to collect contents.
5. The following RT temperatures and times are recommended.
   (a). Oligo(dT)$_{20}$ based cDNA synthesis: Incubate at 37 °C for 50 minutes.
   (b). Random Hexamer based cDNA synthesis: Incubate at room temperature (22-24°C) for 5 minutes. Then transfer the tubes to 37 °C and incubate for 50 minutes.

   * For mRNA targets <1.5 kb, 30 minutes of incubation at 37 °C is an optional choice.
   ** Note: RT temperature may be optimized between 37 and 42 °C.*

6. Inactivate RT by heating at 85°C for 5 min (*optional*). This is recommended when the cDNA synthesis reaction uses less than 10 ng of RNA input.
7. After completion of cDNA synthesis, use 1-2 µl of the first strand reaction out of the 20 µl for PCR amplification. Store the remaining first strand cDNA sample at -20 °C until use**.**

Helpful guidelines

No RT-control: For an accurate gene expression profile study by RT-qPCR (especially rare copy gene quantitation), it is important to include a "no RT" control reaction in the experimental design. For example, if you start with 1 µg of total RNA for cDNA synthesis, use 1 µg of total RNA as a template for the no RT control. Any signal from the no RT control reaction may be attributed to genomic DNA contamination.

DNA digestion of RNA: If amplification products are detected from the PCR reaction in the absence of reverse transcriptase, it may be necessary to remove residual genomic DNA from the RNA sample. Assemble the reaction, incubate at room temperature for 10 minutes, add EDTA (final conc 2.5 mM), and then inactivate DNase I by incubation at 65°C for 15 minutes. This mixture can be directly used for cDNA synthesis.

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Components	Rxn
Total RNA (1-5 µg)	x µl
10X Reaction Buffer (200 mM Tris-Cl(pH 8.4), 500 mM KCl, 20 mM $MgCl_2$)	1 µl
DNaseI (RNase-free grade)	1 unit
Nuclease-free water	to 10 µl