GeneCopoeia™
Expressway to Discovery

All-in-One™ First-Strand cDNA Synthesis Kit

For reliable first-strand cDNA synthesis from all RNA sources

Cat. No. **QP006**（Old Cat. No. AORT-0020, 20 synthesis reactions）
Cat. No. **QP007**（Old Cat. No. AORT-0060, 60 synthesis reactions）

User Manual

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USER MANUAL

All-in-One™ First-Strand cDNA Synthesis Kit

I. Description

The All-in-One™ First-Strand cDNA Synthesis Kit includes a reverse transcriptase and a specialized set of reagents designed to yield first-strand cDNA that is optimal for gene cloning, cDNA library creation and quantitative PCR amplification. A robust experimental design delivers a universal kit that is suitable for first-strand cDNA synthesis from almost any source of RNA.

The kit uses Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (M-MLV RTase (H–)) which is an RNA-dependent DNA polymerase used in cDNA synthesis with long RNA templates (>13kb). 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 required for producing long cDNAs. RNase H minus RT enables preparation of long cDNAs and libraries containing a high percentage of full-length cDNA.

II. Related Products

GeneCopoeia offers comprehensive solutions for studying miRNA. A careful process of co-development ensures that all parts work well together while providing robust and reproducible results.

Product	Description
All-in-One™ qPCR Mix	SYBR Green-based real-time quantitative PCR Mix
All-in-One™ qPCR Primers	Validated, gene-specific primers ensure specificity and sensitivity
RNAzol® RT RNA Isolation Reagent	Easy isolation of mRNA, microRNA and total RNA
ExProfile™ Gene qPCR Arrays	For expression profiling of pre-defined or customized sets of genes in various tissues or cells

All-in-One™ miRNA qRT-PCR Detection Kits	Accurately quantify miRNA expression
All-in-One™ miRNA qPCR Primers	Validated for robust, reproducible and reliable quantitation of miRNA activity
miProfile™ miRNA qPCR Arrays	For expression profiling of pre-defined or customized sets of miRNAs in various tissues or cells

III. Contents and Storage

Contents and storage recommendations for the All-in-One™ First-Strand cDNA Synthesis Kit (**Cat. Nos. QP006 and QP007**) are provided in the following table.

Contents	Quantity	Storage temperature/ conditions
200 U/µl M-MLV Reverse Transcriptase(RNase H-)	1 × 20 µl 3×(1 × 20)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
5 × RT Reaction Buffer	1 × 100 µl 3×(1 ×100)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
25 U/µl RNase Inhibitor	1 × 20 µl 3×(1 × 20)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
25 mM dNTP	1 × 20 µl 3×(1 × 20)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
60 µM Oligo $(dT)_{18}$	1 × 20 µl 3×(1 × 20)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
250 µM Random Primer	1 × 20 µl 3×(1 × 20)µl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.
dd H_2O (RNase and DNase free)	1 × 1 ml 3×(1 ×1) ml	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/ thawing.

IV. Preparation

Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

RNA Sample Preparation

When working with RNA it is important to avoid RNases in your solutions, consumables and labware. When preparing your RNA samples, always wear a mask and disposable gloves in all procedures. Follow the described procedures you are using for RNA extraction carefully. Ready-to-use solutions that are RNase-free can be purchased. Alternatively treat solutions with diethyl pyrocarbonate (DEPC) and then autoclave. RNases on labware can also be inactivated by DEPC treatment or by baking at 250°C for 3 hours. Use DEPC to treat all microcentrifuge tubes, pipettes and pipette tips (if not RNase free) and then autoclave to deactivate RNases. RNase-free consumables are available for purchase from many commercial sources.

IMPORTANT NOTES:

1. Store kit at -20°C. Avoid storage or leaving reagents at 4°C or room temperature.
2. Mix reagents thoroughly by gently inverting tubes several times avoiding bubbles and then briefly centrifuge before use.
3. Set up all reactions on ice to reduce risk of RNA degradation.
4. Read all procedures before setting up RT reaction.

V. Procedure

1. Thaw all the reagents needed for RNA reverse transcription from the All-in-One™ First-Stand cDNA Synthesis Kit. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Prepare the RNA-Primer Mix: Add the following reagents into an RNase-free reaction tube which has been pre-cooled on ice. The final volume should be 13μl.

Reagents	Volume	Final concentration
Total RNA or polyA RNA		1 μg or 10 ng
250 μM Random Primer	1 μl	10 μM
60 μM Oligo $(dT)_{18}$	1 μl	2.4 μM
ddH_2O (RNase/DNase free)	to **13 μl**	

Notes:

- The amount of RNA in the table is the recommended amount. The total RNA may be adjusted to between **10 ng ~ 5 μg**, and the purified poly A RNA between **1 ng ~ 100 ng**.
- Please choose one of the RT Primers based on the experimental design. The reverse transcription will begin at the polyA tail if using the Oligo(dT)$_{18}$. It will begin at many different RT sites throughout the RNA if using the Random Primer. **In order to obtain higher efficiency of RT reaction, using both the Random Primer and the Oligo(dT)$_{18}$ is recommended.**
- In addition, you can also use the Sequence-specific Primer designed by yourself in RT reaction.

3. Denature RNA: Mix the reaction solution well. Spin down briefly. Heat the RNA-Primer mix at 65°C for 10 minutes, and then cool it down immediately on ice.

4. Prepare RNA reverse transcription reaction: Add the following reagents into the RNA-Primer mix reaction tube which has been cooled on ice. The final volume should be 25 μl.

Reagents	Volume	Final concentration
RNA-Primer Mix	13 μl	
5 × RT Reaction Buffer	5 μl	1×
25 mM dNTP	1 μl	1 mM
25 U/μl RNase Inhibitor	1 μl	1 U/μl
200 U/μl M-MLV RTase	1 μl	8 U/μl
ddH_2O (RNase/DNase-free)	to **25 μl**	

5. Reverse Transcription Reaction: Mix reaction solution well. Spin down briefly. Incubate the reaction solution at 37°C for 60 minutes if using both the Random Primer and the Oligo(dT)$_{18}$, or incubate at 42°C for 60 minutes if using sequence-specific primer.

6. Terminate the reaction by heating at 85°C for 5 minutes and then store at -20°C.

7. The cDNA reaction product can be used directly in the next step without being purified. A volume of 0.5 μl ~ 2 μl of undiluted cDNA is recommended for standard 25 μl PCR reactions. If performing quantitative PCR, it is recommended to do a **1:5 ~ 1:20** dilution of the cDNA and add a volume of 2 μl for each 20 μl qPCR reaction.

VI. Example

Objective: The reverse transcription efficiency of the All-in-One™ First Strand Synthesis Kit is assessed by examining the amplification results of different genes or gene regions using the oligo(dT) synthesized cDNA prepared from the All-in-One™ First-Strand cDNA Kit.

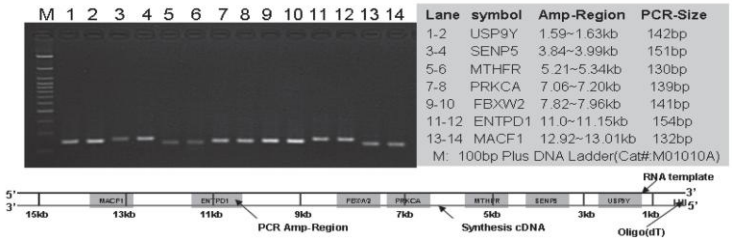

Figure1. Efficient cDNA synthesis by All-in-One™ First-Strand cDNA Synthesis Kit. Total RNA isolated from human placenta was used as template RNA in reverse transcription reactions using the All-in-One™ First-Strand cDNA Synthesis Kit together with the oligo(dT) primer. The synthesized cDNA was then used to amplify different gene regions by quantitative PCR using the All-in-One™ qPCR Mix (GeneCopoeia Cat No.QP001). The positive amplification results of MACF1 indicate that up to a 13 kb RNA sequence was reversed transcribed.

VII. Trouble Shooting Guide

Little or no RT-PCR product	**RNA template degradation** • The quality of the RNA is the key factor for cDNA synthesis. Follow the RNA isolation kit procedure carefully, always wearing a lab coat, gloves and mask when working with RNA and use RNA-Grade reagents and materials. Check the RNA quality by RNA electrophoresis in a denaturing gel. **An inhibitor was present in the RNA template** • Trace amounts of inhibitor such as guanidine salts in the RNA template can inhibit the cDNA synthesis. Re-precipitate the RNA with ethanol and wash the pellet with 75% ethanol. **A G-C rich template or secondary structure of the amplification product is obstructing the reaction** • Prepare the RNA-Primer Mix before the RT step. Then add a PCR enhancing reagent such as DMSO, betaine, etc. in the PCR reaction.
PCR product is longer than expected	• Genomic DNA was present. Perform a DNase I digest before the RT step or design intron-spanning or flanking primers to avoid co-amplification of genomic DNA. • The wrong product was amplified. Optimize the PCR reaction conditions.

VIII. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of All-in-One™ First-Strand cDNA Synthesis Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

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