SureScript™ First-Strand cDNA Synthesis Kit

For reliable first-strand cDNA synthesis from all RNA sources

Cat. No. **QP056** (20 synthesis reactions)
Cat. No. **QP057** (60 synthesis reactions)

User Manual

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USER MANUAL

SureScript™ First-Strand cDNA Synthesis Kit

I. Description

The SureScript™ First-Strand cDNA Synthesis Kit uses a genetically engineered reverse transcriptase. It reduces RNase H activity and increases thermostability. This enzyme can synthesize the first-strand cDNA at a higher temperature than the traditional M-MLV RTase, providing higher specificity and yield of the cDNA. The synthetic cDNA product 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.

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
BlazeTaq™ SYBR® Green qPCR Mix 2.0	SYBR Green-based real-time quantitative PCR Mix
BlazeTaq™ One-Step SYBR® Green RT-qPCR Kit	SYBR Green-based One-Step RT-qPCR Kit
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 SureScript™ First-Strand cDNA Synthesis Kit are provided in the following table.

For kits with the catalog numbers **QP056** and **QP057**

Catalog Number	Contents	Quantity	Storage temperature/ conditions
QP056-01	SureScript RTase Mix (20×)	1 × 20 µl 3 × (1 × 20 µl)	-20°C (Stable for at least 12 months). Avoid repeated freezing/ thawing.
QP056-02	SureScript RT Reaction Buffer (5×)*	1 × 80 µl 3 × (1 × 80 µl)	-20°C (Stable for at least 12 months). Avoid repeated freezing/ thawing.
QP006-07	ddH_2O (RNase and DNase free)	1 × 1 ml 3 × (1 × 1 ml)	–20°C (Stable for at least 12 months). Avoid repeated freezing/ thawing.

*__SureScript RT Reaction Buffer (5×)__ contains the remaining reaction components: reaction buffer, dNTPs, oligo $(dT)_{18}$ and random primers.

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 SureScript™ First-Stand cDNA Synthesis Kit. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Prepare RNA reverse transcription reaction solution according to the table below, The final volume should be 20 µl. Mix the reaction solution well. Spin down briefly.

Reagents	Volume	Final concentration
SureScript RTase Mix (20×)	1 µl	1 ×
SureScript RT Reaction Buffer (5×)	4 µl	1×
Total RNA or poly A RNA		1 ng or 10 ng
ddH_2O (RNase/DNase free)	to **20 µl**	

Notes:

The sensitivity of this kit can be from 0.1 pg to 10 μg, depending on target abundance and RNA quality. Generally, 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.

RNA template can be quantitated using UV absorbance at 260 nm. RNA quality can be analyzed using a bioanalyzer or agarose gel electrophoresis.

3. The following method for programming the reverse transcription reaction is recommended:

Temperature	Time
25℃	5 min
42℃	15 min*
85℃	5 min
4℃	hold

*Generally, 15 minutes is sufficient for the reverse transcription reaction of most RNA templates. For more complex or longer templates, the reaction time can be prolonged appropriately according to the actual situation, generally not more than 60 minutes.

4. 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. 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.

VII. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of SureScript™ 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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