Certificate of Analysis

GoScript™ Reverse Transcriptase

Part# 9PIA500

Revised 10/16

Cat.#	Size
A5002	10 reactions
A5003	100 reactions
A5004	500 reactions

Description: GoScript™ Reverse Transcriptase[a] utilizes M-MLV and state-of-the-art buffer technology designed for qPCR to deliver robust, reliable cDNA synthesis of a full range of rare and abundant transcripts, even in the presence of inhibitors. GoScript™ Reverse Transcriptase[a] is qualified for use in qPCR, including GoTaq® qPCR and Plexor® qPCR systems for performing RT-qPCR.

GoScript™ Reverse Transcriptase is provided in quantities sufficient for 10–500 first-strand cDNA synthesis reactions of 20µl each. **Additional components are required to perform the reactions.** Contains one of the following:

Cat.# A5002

Part No.	Component	Size
A501A	GoScript™ Reverse Transcriptase	10µl
A500A	GoScript™ 5X Reaction Buffer	100µl
A351B	$MgCl_2$, 25mM	750µl

Cat.# A5003

Part No.	Component	Size
A501C	GoScript™ Reverse Transcriptase	100µl
A500C	GoScript™ 5X Reaction Buffer	600µl
A351H	$MgCl_2$, 25mM	1.2ml

Cat.# A5004

Part No.	Component	Size
A501D	GoScript™ Reverse Transcriptase	500µl
A500D	GoScript™ 5X Reaction Buffer	2 × 1.25ml
A351H	$MgCl_2$, 25mM	3 × 1.2ml

Concentration: GoScript™ Reverse Transcriptase is supplied at a concentration of 160u/µl.

Storage Conditions: Store at –20°C.

Expiration Date: See the product label for the expiration date.

Biological Source: Recombinant *E. coli* strain.

Quality Control Assays

This lot passes the following quality control specifications:

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C.

First-Strand cDNA Synthesis: First-strand cDNA is synthesized using 1.25µl of GoScript™ enzyme, 1µg of a 6.5kb control RNA, oligo(dT) primer and radiolabeled dNTP. The specification is the conversion of >12% of mRNA to cDNA. Full-length cDNA is observed by gel electrophoresis and autoradiography.

Amplification:

RT-PCR: When approximately 100 copies of a 1.2kb Kanamycin Positive Control RNA (Cat.# C1381) are reverse transcribed at 42°C and amplified, the result is a clear, discrete 323bp DNA product as visualized on an agarose gel by ethidium bromide staining.

RT-qPCR: One microgram of total RNA positive control (Part# C199A) is reverse transcribed at 42°C for 1 hour. The resulting cDNA is serially diluted over six orders of magnitude and amplified using GoTaq® qPCR Master Mix, 2X (Part# A600A), to generate a standard curve. The standard curve has an $R^2 \geq 0.990$.

Signed by:

R. Wheeler, Quality Assurance

[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

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Usage Information

I. First-Strand cDNA Synthesis

The following procedure can be used to convert up to 5µg of total RNA or up to 500ng of poly(A) RNA into first-strand cDNA.

1. Mix and briefly centrifuge each component before use. Combine the following:

Component	Volume
Experimental RNA (up to 5µg/reaction)	Xµl
Primer [Oligo(dT)$_{15}$ (0.5µg/reaction) and/or Random Primer (0.5µg/reaction) or gene-specific primer (10–20pmol/reaction)]	Xµl
Nuclease-Free Water	Xµl
Final volume	5µl

2. Heat in a 70°C heat block for 5 minutes. Immediately chill in ice water for at least 5 minutes. Centrifuge 10 seconds in a microcentrifuge. Store on ice until reverse transcription mix is added.

3. Prepare the reverse transcription reaction mix, 15µl for each cDNA reaction. Combine on ice, in the order listed.

Component	Volume
GoScript™ 5X Reaction Buffer	4.0µl
$MgCl_2$ (final concentration 1.5–5.0mM)[1]	1.2–6.4µl
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)[2]	1.0µl
Recombinant RNasin® Ribonuclease Inhibitor (optional)	20units
GoScript™ Reverse Transcriptase	1.0µl
Nuclease-Free Water (to a final volume of 15µl)	Xµl
Final volume	15µl

[1]Mg^{2+} concentration should be optimized to 1.5–5.0mM ($MgCl_2$ provided at 25mM).
[2]If isotopic or nonisotopic incorporation is desired for monitoring first-strand cDNA synthesis, α[^{32}P]-dCTP or other modified nucleotides may be supplemented into the PCR Nucleotide Mix. See Section 4.D of TM316, for analysis suggestions.

4. Combine 15µl of reverse transcription mix with 5µl of RNA and primer mix.

5. Anneal in a heat block at 25°C for 5 minutes.

6. Extend in a heat block at 42°C for up to one hour.

   Reactions can be stopped at this point for analysis of the cDNA or may be frozen for long-term storage.

7. Inactivate Reverse Transcriptase: Before proceeding with qPCR, inactivate the reverse transcriptase in a heat block at 70°C for 15 minutes.

II. cDNA Quantification Using qPCR

cDNA synthesized using GoScript™ Reverse Transcriptase can be amplified and quantified using the GoTaq® qPCR Master Mix or the Plexor® qPCR Systems. cDNA samples may be used directly or diluted prior to amplification. As a starting point for dilution, dilute sample and reference standard cDNA reactions 1:10, then add 5µl of these diluted reactions to the reaction mix. For additional information, refer to the *GoTaq® qPCR Master Mix Technical Manual,* #TM318, or to the *Plexor® qPCR System Technical Manual*, #TM262.

1. Heat inactivate the reverse transcription reaction.

2. Proceed directly to GoTaq® or Plexor® qPCR target-specific quantitative analysis of the cDNA.

3. Alternatively, heat-inactivated reactions may be stored frozen for future use.

4. GoTaq® and Plexor® qPCR accommodate the addition of up to 20% of the total reaction volume as template GoScript™ Reverse Transcriptase reaction volume.

5. The cDNA may be added directly to the qPCR, as undiluted reverse transcription reaction product, or it may be diluted.

6. The dilution factor must be experimentally determined to be appropriate for the amount of RNA template mass and proportional reverse-transcript representation in the cDNA sample.

7. Generally, for cDNA quantitation, using the default analysis settings of many real-time instruments, the amount of cDNA used as template in GoTaq® qPCR should not exceed that correlating with a proportional 100ng of input total RNA. The cDNA generated from highly abundant transcripts can be detected in less than 1pg of total RNA.

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