

Certificate of Analysis

GoScript™ Reverse Transcription Mix, Random Primers:

Cat.#	Size
A2800	50 reactions
A2801	100 reactions

Part# 9PIA280
Revised 12/16

Description: The GoScript™ Reverse Transcription Mix, Random Primers is a convenient mix that includes the GoScript™ Enzyme Mix (GoScript™ Reverse Transcriptase and Recombinant RNasin® Ribonuclease Inhibitor) and the GoScript™ Reaction Buffer (Random Primers, MgCl₂, and dNTPs) which have been optimized to provide efficient synthesis of first-strand cDNA, in preparation for qPCR amplification. The components of the GoScript™ Reverse Transcription Mix can be used to reverse transcribe RNA templates starting with total RNA, poly(A) + mRNA or synthetic transcript RNA.

Contains one of the following:

Cat.# A2800

Part No.	Component	Size
A276A	GoScript™ Enzyme Mix	100µl
A278A	GoScript™ Reaction Buffer, Random Primers	200µl
P119A	Nuclease-Free Water	1.25ml

Cat.# A2801

Part No.	Component	Size
A276A	GoScript™ Enzyme Mix	2 × 100µl
A278A	GoScript™ Reaction Buffer, Random Primers	2 × 200µl
P119A	Nuclease-Free Water	2 × 1.25ml

Storage Conditions: Store at -30°C to -10°C.

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



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Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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Quality Control Assays

Two Step RT-qPCR: The GoScript™ Reverse Transcription Mix, Random Primers is tested in two-step RT-qPCR. cDNA synthesis is performed using the the GoScript™ Reverse Transcription Mix, Random Primers, GoScript™ Enzyme Mix and human total RNA template amounts from 10pg to 1µg. The resulting cDNA products are used as templates for qPCR using GoTaq® qPCR Master Mix (A6001). All cDNA amplifications must yield a single product with the correct dissociation temperature. The standard curve for the qPCRs must have a slope of -3.3 ± 0.3 and an $R^2 \geq 0.980$.

No-Template Controls: Three of four no-RNA template controls must contain no detectable amplification product.

Signed by:

R. Wheeler, Quality Assurance

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All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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GoScript™ Reverse Transcription Mix, Random Primer Protocol

The following protocol can be used to convert a broad range of RNA input quantities, up to 5µg of total RNA or 500ng of poly(A)+RNA, into full-length, random primer-primed first-strand cDNA products.

Materials to be Supplied by User

- nuclease-free, low retention qPCR-compatible reaction tubes or plates
- pipettes and sterile, aerosol-resistant tips
- experimental and reference RNA samples
- thermal cycler or 25°C, 42°C and 70°C controlled-temperature heat blocks
- ice or 4°C blocks

First-Strand cDNA Synthesis

1. Thaw the GoScript™ Reverse Transcription Mix components on ice, mix gently and centrifuge briefly.
2. Prepare 10µl of GoScript™ Reverse Transcription Mix for each cDNA reaction by combining components on ice in the order listed. Mix gently by pipetting after each addition.

Component	Volume
Nuclease-Free Water	4µl
GoScript™ Reaction Buffer, Random Primer	4µl
GoScript™ Enzyme Mix	<u>2µl</u>
Final Volume	10µl

3. Gently mix the GoScript™ Reverse Transcription Mix by pipetting or vortexing.
4. **Optional:** To denature RNA with high degrees of secondary structure (>60% GC content), incubate RNA samples at 70°C for 5 minutes, then immediately chill in ice water for at least 5 minutes. Centrifuge in a microcentrifuge for 5 seconds.
5. Combine 10µl of GoScript™ Reverse Transcription Mix and up to 10µl of RNA sample in each reaction tube or well of a reaction plate on ice as shown below.

Component	Volume
GoScript™ Reverse Transcription Mix	10µl
Experimental or Reference RNA	<u> </u> µl
Nuclease-Free Water	to a final volume of <u>20µl</u>
Final Volume	20µl

6. Mix each reverse transcription reaction by pipetting. Close or seal the reaction tubes or wells.
7. Incubate the reverse transcription reactions in controlled-temperature blocks or in a programmed thermal cycler.

Step	Temperature	Time	Number of Cycles
Anneal primer*	25°C	5 minutes	1 cycle
Extension	42°C ¹	60 minutes	1 cycle
Inactivation	70°C ²	15 minutes	1 cycle
Hold	4°C	∞	1 cycle

* Optional

¹ The extension temperature may be optimized between 37–55°C.

² Higher temperatures up to 95°C for 5 minutes may be used for the inactivation step.

8. Store the reaction products at 4°C in the reaction tubes or wells for immediate analysis (up to 24 hours) or at –20°C for long-term storage.