Certificate of Analysis

GoScript™ Reverse Transcription Mix, Oligo(dT)

Cat.#	Size
A2790	50 reactions
A2791	100 reactions

Part# 9PIA279
Revised 12/16

Description: The GoScript™ Reverse Transcription Mix, Oligo(dT) is a convenient mix that includes the GoScript™ Enzyme Mix (GoScript™ Reverse Transcriptase and Recombinant RNasin® Ribonuclease Inhibitor) and the GoScript™ Reaction Buffer (Oligo(dT), $MgCl_2$, 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 total RNA, poly(A) + mRNA or synthetic transcript RNA.

Contains one of the following:

AF9PIA279 1216A279

Cat.# A2790

Part No.	Component	Size
A276A	GoScript™ Enzyme Mix	100µl
A277A	GoScript™ Reaction Buffer, Oligo(dT)	200µl
P119A	Nuclease-Free Water	1.25ml

Cat.# A2791

Part No.	Component	Size
A276A	GoScript™ Enzyme Mix	2 × 100µl
A277A	GoScript™ Reaction Buffer, Oligo(dT)	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.

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

Quality Control Assays

Two Step RT-qPCR: The GoScript™ Reverse Transcription Mix, Oligo(dT) is tested in two-step RT-qPCR. cDNA synthesis is performed using the the GoScript™ Reverse Transcription Mix, Oligo(dT), 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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Promega

GoScript™ Reverse Transcription Mix, Oligo(dT) 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, oligo(dT)-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, Oligo(dT)	4µl
GoScript™ Enzyme Mix	2µl
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	__µl
Nuclease-Free Water	to a final volume of 20µl
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.

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