Certificate of Analysis

GoScript™ MDx Reverse Transcriptase:

Part# 9PID120
Printed 6/24

Cat.# D1201 is sufficient for 300 reactions (50ku). Contains:

Part No.	Component	Size
D112A	$MgCl_2$, 25mM	2 × 1.2ml
A500C	GoScript™ 5X Reaction Buffer	2 × 600µl
D120A	GoScript™ MDx Reverse Transcriptase	40µl

Description: This enzyme is a high-concentration formulation of GoScript™ MDx Reverse Transcriptase, a Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, that delivers robust, reliable complementary DNA (cDNA) synthesis. GoScript™ MDx Reverse Transcriptase is qualified for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and manufactured under Current Good Manufacturing Practices.

Enzyme Concentration: 1,250u/µl.

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

Source: Recombinant *E. coli* strain.

Storage Conditions: See the product label for storage recommendations.

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Quality Control Assays

Test			Specification	Result
Concentration			1,250u/µl (1,000–1,500u/µl) 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.	Pass
Purity	DNA Contamination	Bacterial DNA	One unit of enzyme contains less than 10 copies of bacterial genomic DNA determined by quantitative amplification of a 16s rRNA gene.	Pass
		Fungal DNA	One unit of enzyme contains less than 1 genome equivalent of fungal genomic DNA by quantitative amplification of a 18S rRNA gene.	Pass
		Mammalian DNA	One unit of enzyme contains less than 1 genome equivalent of mammalian gDNA by quantitative amplification of mitochondrial genomic DNA.	Pass
	Nuclease Contamination	DNase	Using DNaseAlert, mean percent released of each test sample ≤1.75%.	Pass
		RNase	Using RNaseAlert, mean percent positive of each test sample ≤0.5%.	Pass
	Protein Purity	Electrophoretic Purity	>90% pure when electrophoresed on a 4–20% SDS-PAGE gel, stained with Coomassie® blue dye	Pass
Function			When used in an RT-PCR followed by qPCR, r^2 ≥0.980; slope ≥–3.70 and ≤–3.20; intercept ≥36 and ≤39.	Pass

Signed by:

R. Wheeler, Quality Assurance

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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Usage Information

1. First-Strand cDNA Synthesis

The following procedure can be used to convert total RNA into first-strand complementary DNA (cDNA).

1. Mix and briefly centrifuge each component before use. For each cDNA synthesis reaction, combine the following:

Component	Volume
RNA (up to 5µg/reaction)	___µl
Primer Oligo $(dT)_{15}$ (0.5µg/reaction) and/or Random primer (0.5µg/reaction) or Gene-specific primer (10–20pmol/reaction	___µl
nuclease-free water	___µl
Final volume	**5µl**

2. Heat in a 70°C heating 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 the following on ice:

Component	1X Reaction Volume	10X Reactions Volume
GoScript™ 5X Reaction Buffer	4.0µl	40µl
$MgCl_2$ (final concentration 1.5–5.0mM)[1]	1.2–6.4µl	12–64µl
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)	1.0µl	10µl
optional: RNasin® Plus Ribonuclease Inhibitor	20 units	200 units
GoScript™ MDx Reverse Transcriptase[2]	0.128µl	1.28µl
nuclease-free water	__µl (To a 15µl final volume)	__µl (To a 150µl final volume)
Final volume (per reaction)	**15µl**	**15µl**

[1]Mg^{2+} concentration should be optimized to 1.5–5.0mM ($MgCl_2$ provided at 25mM).
[2]The high-concentration GoScript™ MDx Reverse Transcriptase (Cat.# D1201) enzyme is too concentrated to prepare a reverse transcription reaction mix for one 15µl cDNA synthesis reaction as described. The GoScript™ MDx enzyme is intended for preparing at least ten separate cDNA synthesis reactions or for alternative applications where a greater reverse transcriptase activity is required.

4. Combine 15µl of reverse transcription mix with 5µl of RNA and primer mix.
5. Anneal in heating block at 25°C for 5 minutes.
6. Extend in a heating block at 42°C for up to 1 hour.
7. Inactivate the reverse transcriptase before proceeding with qPCR by incubating the reaction mix in a heating block at 70°C for 15 minutes.

2. Guidance for cDNA Amplification by PCR

1. Heat-inactivate the reverse transcription reaction (see Section 1, Step 7).
2. Add the cDNA directly to the PCR amplification as undiluted reverse transcription reaction product, or dilute the reaction. If diluted, experimentally determine the dilution factor.
3. You can add up to 20% of the total PCR amplification volume as template when using GoTaq® qPCR master mix.

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