GoScript™ Reverse Transcription System

INSTRUCTIONS FOR USE OF PRODUCTS A5000 AND A5001.

Quick PROTOCOL

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**

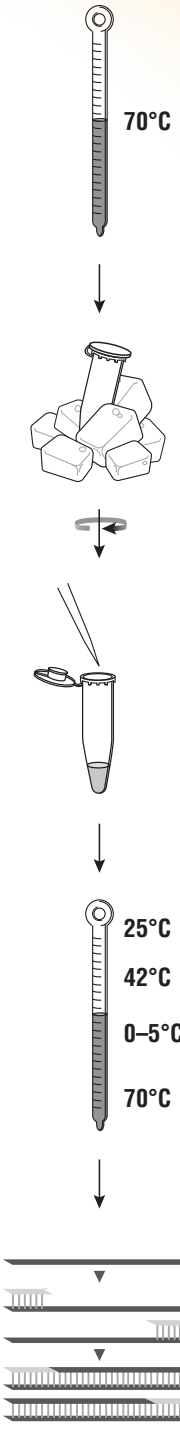
[1]Mg^{2+} concentration should be optimized to 1.5–5.0mM ($MgCl_2$ provided at 25mM).

[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, 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.

(continued)



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cDNA Quantification Using qPCR

1. Quantify specific targets in samples of undiluted or diluted cDNA using GoTaq® qPCR Master Mix.
2. Alternatively, add a diluted sample of cDNA, as determined to be optimal (up to 20% of the reaction volume or 100ng of input RNA).

See additional protocol information in the *GoScript™ Reverse Transcription System Technical Manual,* #TM316, available at: **www.promega.com/tbs**

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