Reverse Transcription System

INSTRUCTIONS FOR USE OF PRODUCT A3500.

Quick PROTOCOL

Reverse Transcription Protocol

Reverse Transcription Reaction (First-Strand cDNA Synthesis)

1. Place 1µg (2µl) of 1.2kb Kanamycin Positive Control RNA, poly(A)+ mRNA or total RNA in a microcentrifuge tube, and incubate at 70°C for 10 minutes. Centrifuge briefly in a microcentrifuge, then place on ice.
2. Prepare a 20µl reaction by adding the following reagents in the order listed (this reaction can be scaled up or down, depending on the amount of RNA):

Component	Amount
$MgCl_2$, 25mM	4µl
Reverse Transcription 10X Buffer	2µl
dNTP Mixture, 10mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl
AMV Reverse Transcriptase (High Conc.)	15u
$Oligo(dT)_{15}$ Primer OR Random Primers	0.5µg
1.2kb Kanamycin Positive Control RNA (2µl) OR poly(A)+ mRNA OR total RNA	1µg
Nuclease-Free Water to a final volume of	20µl

3. When using $Oligo(dT)_{15}$ Primer, incubate the reaction at 42°C for 15 minutes. When using Random Primers, incubate the reaction at room temperature for 10 minutes, then incubate at 42°C for 15 minutes.
4. Heat the sample at 95°C for 5 minutes, then incubate at 0–5°C for 5 minutes.

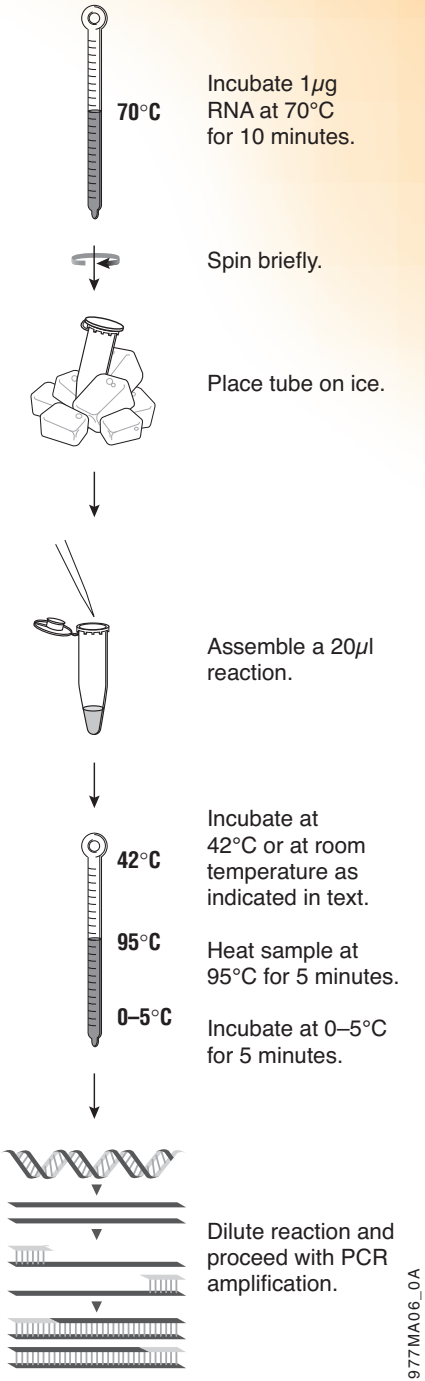
Dilution of the Reaction for Amplification

1. Dilute the first-strand cDNA synthesis reaction to 100µl with TE buffer or Nuclease-Free Water.
2. Prepare a 100µl PCR amplification mix by combining the following reagents. Template-specific upstream and downstream primers should be used for this reaction.

Component	Amount
first-strand cDNA reaction	10–20µl
dNTP Mixture, 10mM	1.8µl
$MgCl_2$, 25mM	7.5µl
Reverse Transcription 10X Buffer	9.8µl
upstream primer	50pmol
downstream primer	50pmol
Taq DNA polymerase	2.5 units
Nuclease-Free Water to a final volume of	100µl

3. Proceed to thermal cycling according to your own specific experiment.

See additional protocol information in Technical Bulletin #TB099, available online at: **www.promega.com**



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