Access RT-PCR System

INSTRUCTIONS FOR USE OF PRODUCTS A1250, A1260 AND A1280.

Quick PROTOCOL

Assemble Reactions

1. Combine the reagents below in a thin-walled 0.5ml reaction tube on ice.

Reagents	Volume	Final Concentration
Nuclease-Free Water (to a final volume of 50µl)	Xµl	
AMV/*Tfl* 5X Reaction Buffer	10µl	1X
dNTP Mix (10mM each dNTP)	1µl	0.2mM
Downstream primer	50pmol	1µM
Upstream primer	50pmol	1µM
25mM $MgSO_4$	2µl	1mM

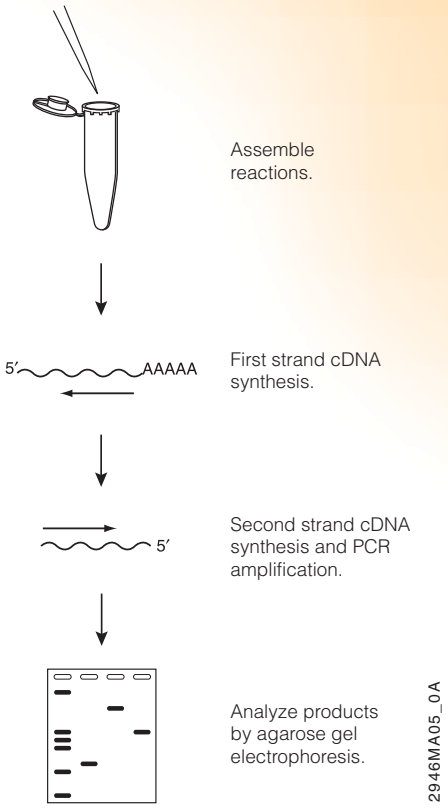
2. Mix by pipetting. Add the remaining components.

AMV Reverse Transcriptase (5u/µl)	1µl	0.1u/µl
Tfl DNA Polymerase (5u/µl)	1µl	0.1u/µl

3. Gently vortex. Initiate the reaction by adding:

RNA template	Yµl	10^3–10^6 copies
	50µl	

4. Overlay the reactions with 1 or 2 drops of mineral oil.



Assemble reactions.

First strand cDNA synthesis.

Second strand cDNA synthesis and PCR amplification.

Analyze products by agarose gel electrophoresis.

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First Strand cDNA Synthesis

These PCR cycling profiles are only a guideline. Cycling conditions should be optimized for each RNA template.

1 cycle	45°C for 45 minutes	reverse transcription
1 cycle	94°C for 2 minutes	AMV RT inactivation and RNA/cDNA/primer denaturation

Second Strand Synthesis and PCR Amplification

40 cycles	94°C for 30 seconds 60°C for 1 minute 68°C for 2 minutes	denaturation annealing extension
1 cycle (optional)	68°C for 7 minutes	final extension
1 cycle	4°C	soak

Analyze 2.5µl of the reaction products by agarose gel electrophoresis. Store the remainder of the reaction at –20°C.

Additional protocol information is available in Technical Bulletin #TB220, available online at: **www.promega.com**

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