TECHNICAL BULLETIN

Access RT-PCR System

Instructions for Use of Products
A1250, A1260 and A1280

Revised 7/24
TB220

Promega

Access RT-PCR System

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Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

Numerous techniques have been developed to measure gene expression in tissues and cells. These include Northern blots, coupled reverse transcription and PCR amplification (RT-PCR), RNase protection assays, in situ hybridization, dot blots and S1 nuclease assays. Of these methods, RT-PCR is the most sensitive and versatile. This technique can be used to determine the presence or absence of a transcript, estimate expression levels and clone cDNA products without the necessity of constructing and screening a cDNA library.

The Access RT-PCR System[a] is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA (1). This one-tube, two-enzyme system provides sensitive, quick and reproducible analysis of even rare RNAs. The system uses AMV Reverse Transcriptase (AMV RT) from Avian Myeloblastosis Virus for first strand DNA synthesis and the thermostable *Tfl* DNA Polymerase from *Thermus flavus* (2) for second strand cDNA synthesis and DNA amplification. The Access RT-PCR System includes an optimized single-buffer system that permits extremely sensitive detection of RNA transcripts without a requirement for buffer additions between the reverse transcription and PCR amplification steps (Figure 1). This simplifies the procedure and reduces the potential for contaminating the samples. In addition, the improved performance of AMV Reverse Transcriptase at elevated temperatures [45°C[a]] in the AMV/*Tfl* 5X Reaction Buffer minimizes problems encountered with secondary structures in RNA.

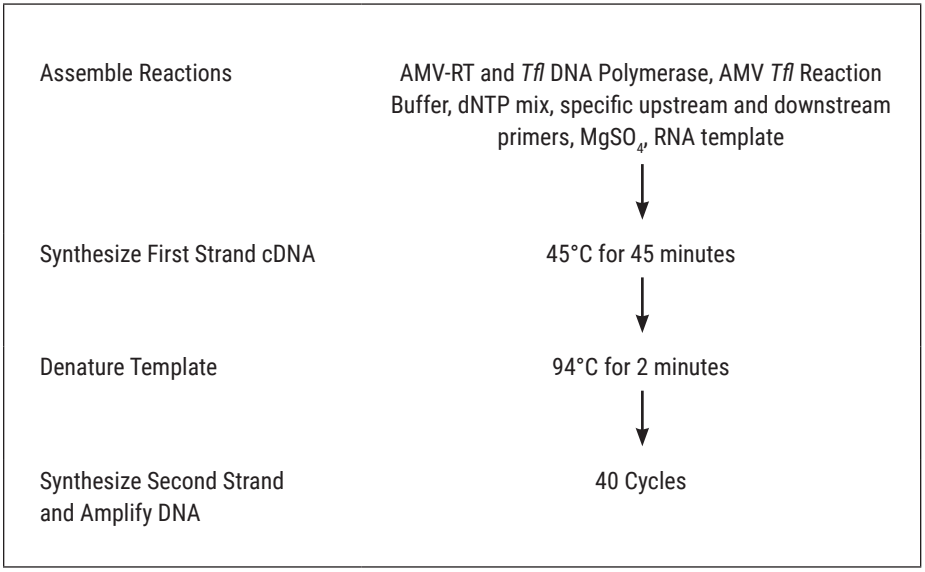

Figure 1. Outline of the Access RT-PCR System protocol.

2. Product Components and Storage Conditions

PRODUCT	CAT.#
Access RT-PCR System	**A1250**

Each system contains sufficient reagents for 100 reactions (50µl), including 25 reactions containing Positive Control RNA and Primers. Includes:

- 500u AMV Reverse Transcriptase, 5u/µl
- 500u *Tfl* DNA Polymerase, 5u/µl
- 1ml AMV/*Tfl* 5X Reaction Buffer
- 1.25ml $MgSO_4$, 25mM
- 100µl dNTP Mixture, 10mM each of dATP, dCTP, dGTP and dTTP
- 50µl Positive Control RNA with Carrier (1.25 attomole/µl)
- 100µl Upstream Control Primer, 15µM
- 100µl Downstream Control Primer, 15µM
- 13ml Nuclease-Free Water

PRODUCT	CAT.#
Access RT-PCR System	**A1280**

Each system contains sufficient reagents for 500 reactions (5 kits × 100 reactions, 50µl each), including 25 reactions containing Positive Control RNA and Primers (provided in one of the kits).

PRODUCT	CAT.#
Access RT-PCR Introductory System	**A1260**

Each system contains sufficient reagents for 20 reactions (50µl) and includes reagents for positive control reactions (Positive Control RNA and Primers). Includes:

- 100u AMV Reverse Transcriptase, 5u/µl
- 100u *Tfl* DNA Polymerase, 5u/µl
- 1ml AMV/*Tfl* 5X Reaction Buffer
- 1.25ml $MgSO_4$, 25mM
- 20µl dNTP Mixture, 10mM each of dATP, dCTP, dGTP and dTTP
- 50µl Positive Control RNA with Carrier (1.25 attomole/µl)
- 100µl Upstream Control Primer, 15µM
- 100µl Downstream Control Primer, 15µM
- 13ml Nuclease-Free Water

Storage Conditions: Store all system components at –30°C to –10°C. For long-term storage, the Positive Control RNA with Carrier may be stored at less than –65°C. See the expiration date on the kit label.

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3. Optimizing RT-PCR

3.A. RNA Template

Successful reverse transcription is dependent on the integrity and purity of the mRNA used as the template. Procedures for creating and maintaining an RNase-free environment are described in reference 3. Use sterile tubes, pipette tips, gloves and diethyl pyrocarbonate (DEPC)-treated water. When isolating RNA from samples high in ribonuclease activity, use of a ribonuclease inhibitor such as Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511) is recommended.

For the routine and rapid purification of total RNA from eukaryotic sources, we recommend the SV Total RNA Isolation System (Cat.# Z3100).

Poly(A)+ RNA can be efficiently isolated from total RNA using the PolyATtract® mRNA Isolation Systems (Cat.# Z5200, Z5300) or directly from eukaryotic sources using the PolyATtract® System 1000 (Cat.# Z5420, Z5400). These systems enable the isolation of total or poly(A)+ RNA from crude cell or tissue lysates and result in RNA preparations sufficiently pure for use in the Access RT-PCR System.

For optimal results using this system, the RNA template, whether a total RNA preparation, an mRNA population or a synthesized RNA transcript, should be DNA-free. The *Tfl* DNA Polymerase included with this system has no reverse transcriptase activity under the standard reaction conditions (1), but amplification product will be generated out of these reactions if trace amounts of DNA with similar sequences are present in the template preparation.

The minimum amount of RNA that can be amplified using RT-PCR is both template- and primer-dependent. For the Positive Control RNA provided, the minimum amount of RNA required is 10^3 molecules (1.66 zeptomoles, 1.66×10^{-21} moles) (Figure 2, Panel A). Excellent amplification results can be obtained with the Access RT-PCR System using total RNA template levels in the range of 10pg–1µg per reaction (Figure 2, Panel B), or poly(A)+ RNA template levels in the range of 1pg–100ng.

3.B. Control Reactions

To facilitate optimization and troubleshooting, perform both positive and negative control reactions. For positive control reactions, use the supplied Positive Control RNA with Carrier (see Section 7.A) and the Upstream and the Downstream Control Primers (see Figure 2, Panel A). For a negative control, substitute sterile nuclease-free water for the RNA template in the reaction.

Note: The Upstream and Downstream Control Primers will only amplify the Positive Control RNA.

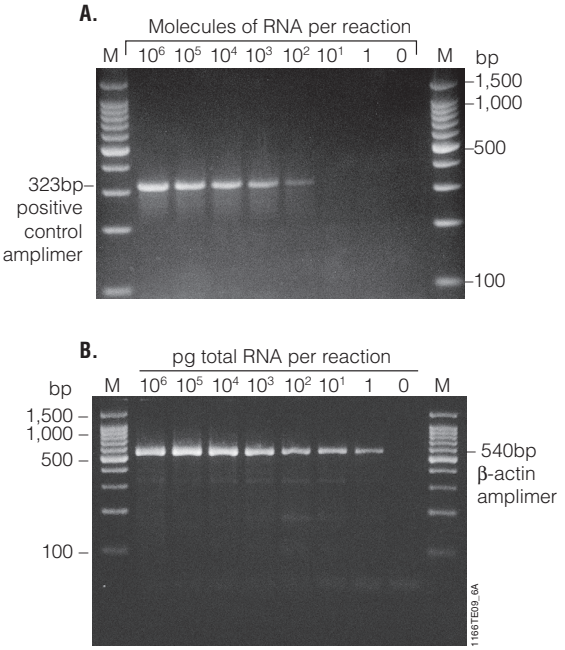

Figure 2. Amplification of specific RNAs using the Access RT-PCR System. Panel A. Serial tenfold dilutions of the Positive Control RNA supplied with the system were prepared in Nuclease-Free Water. RT-PCR reactions containing the indicated amounts of Positive Control RNA were performed as described in Section 4 using the control oligonucleotide primers provided. Equivalent aliquots of each amplification reaction were separated on a 3% NuSieve®/1% agarose gel in 1X TAE buffer containing 0.5µg/ml ethidium bromide. The specific 323bp amplimer is indicated. Lane M, 100bp DNA Ladder (Cat.# G2101). **Panel B**. RT-PCR reactions containing the indicated amounts of mouse liver total RNA were performed as described in Section 4 using oligonucleotide primers specific to the mouse β-actin transcript. The specific 540bp amplimer is indicated. Equivalent aliquots of each amplification reaction were separated on a 3% NuSieve®/ 1% agarose gel in 1X TAE buffer containing 0.5µg/ml ethidium bromide. Lane M, 100bp DNA Ladder (Cat.# G2101).

3.C. Avoiding Contamination of Nucleic Acids

Take care to minimize the potential for cross-contamination between samples and prevent carryover of nucleic acid (RNA and DNA) from one experiment to the next. Use a separate work area and pipettor for pre- and post-amplification steps. Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. Wear gloves and change them often. Use UNG (4) or another sterilization technique to prevent DNA carryover to subsequent reactions.

3.D. Magnesium Concentration

The magnesium requirement of both the AMV Reverse Transcriptase and the *Tfl* DNA Polymerase in the Access RT-PCR System reactions is affected by the final concentration of nucleotides, oligonucleotide primers and template. The magnesium sulfate concentration should be optimized for each experimental target/primer combination. Although 1.0–2.5mM magnesium sulfate is suitable for most applications, titration of the magnesium sulfate concentration can significantly improve the sensitivity, specificity and quality of the reverse transcription and amplification products.

To determine the optimal magnesium concentration for a specific template/ primer combination, prepare a reaction series containing 1.0–3.0mM magnesium sulfate in 0.5mM increments by adding 2, 3, 4, 5 or 6µl of the 25mM Magnesium Sulfate stock to 50µl reactions.

! Vortex the magnesium stock prior to use.

3.E. Primer Design

A specific primer should be used for first strand synthesis. Specific primers anneal only to defined sequences and can be used to synthesize cDNA from particular mRNAs rather than from the entire mRNA population in the sample. To differentiate between amplification of cDNA and amplification of contaminating genomic DNA, primers may be designed to anneal to sequences in exons on opposite sides of an intron. An amplification product derived from genomic DNA will be much larger than the product of the RT-PCR amplification reaction. This size difference not only makes it possible to differentiate the two products by gel electrophoresis, it also favors the synthesis of the smaller cDNA-derived product (PCR favors the amplification of smaller fragments). Regardless of primer choice, the final concentration of the primer in the reaction may need to be optimized. We recommend adding 50pmol of primer (1µM final concentration in reaction) as a starting point for optimization.

3.F. Temperature

The Access RT-PCR System does not require a template denaturation step prior to initiation of the reverse transcription reaction. If desired, a denaturation step may be incorporated by incubating a separate tube containing the primers and RNA template at 94°C for 2 minutes. The template/primer mixture can then be added to the RT-PCR reaction mix for the standard reverse transcription incubation at 45°C.

! Do not incubate the AMV RT at 94°C; it will be inactivated.

AMV Reverse Transcriptase is active in the AMV/*Tfl* 5X Reaction Buffer at temperatures between 37°C and 45°C. We recommend that the reverse transcription reaction be performed at 45°C to minimize the effects of RNA secondary structure and to encourage full-length cDNA synthesis. Following the reverse transcription incubation, we recommend a two-minute incubation at 94°C to denature the RNA/cDNA hybrid and inactivate the AMV Reverse Transcriptase. It has been reported that the AMV Reverse Transcriptase enzyme must be inactivated to obtain high yields of amplification product using thermophilic DNA polymerases such as *Tfl* DNA Polymerase (5,6).

The sequences of the primers are a major consideration in determining the temperature of the PCR amplification cycles. An amplification cycle typically consists of a denaturation step (94°C), a template/primer annealing step (42–60°C) and an extension step (68°C). For primers with a high T_m, it may be advantageous to increase the suggested annealing and extension temperatures. The higher temperature minimizes nonspecific primer annealing, thus increasing the amount of specific product produced. For primers with a low T_m, it may be necessary to decrease the annealing temperature to allow the primer to anneal to the target template.

3.G. Incubation Times and Number of Cycles

Efficient first strand cDNA synthesis can be accomplished in a 15- to 60-minute incubation at 37–45°C. We recommend a 45-minute incubation at 45°C as a general starting point.

Following the first strand cDNA synthesis, the AMV Reverse Transcriptase is inactivated and the RNA/cDNA hybrid denatured using a 2-minute incubation at 94°C. This step leads directly into the second strand cDNA synthesis and PCR amplification phase of the procedure. Most RNA samples can be detected using 40 cycles of amplification. If the target RNA is rare or if only a small amount of starting material is available, it may be necessary to increase the number of cycles to 45 or 50. During the extension step, allow approximately 1 minute for every 1kb of amplimer (minimum extension time = 1 minute). A final 7-minute extension at 68°C improves the quality of the final product by extending truncated product to full-length.

4. Synthesis and Analysis of RT-PCR Products Using the Access RT-PCR System

The reverse transcription and PCR cycling profile provided in Figure 3 should serve as a guideline for initial experiments. These conditions work well for the detection of the 323bp PCR product generated from the Positive Control RNA using the Upstream and Downstream Control Primers provided with the Access RT-PCR System. We recommend optimizing the parameters discussed in Section 3 for each combination of primers and target RNA.

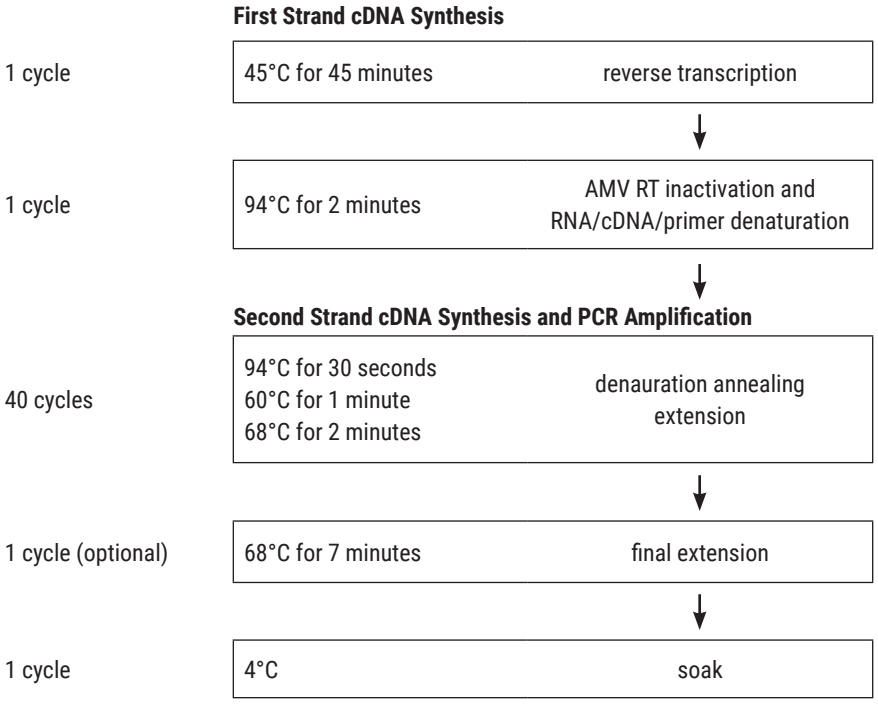

Figure 3. Reverse transcription and PCR cycling profile.

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4.A. Protocol

Materials to Be Supplied by the User

- downstream oligonucleotide primer
- upstream oligonucleotide primer
- nuclease-free light mineral oil (e.g., Sigma Cat.# M5904)

1. Prepare the reaction mix by combining the indicated volumes of the first six components in a thin-walled 0.5ml reaction tube on ice. Mix by pipetting. Add the AMV Reverse Transcriptase and *Tfl* DNA Polymerase to the reaction. Gently vortex the tube for 10 seconds to mix the components. If working with multiple samples, a Master Mix may be assembled on ice by combining appropriate multiples of each of the indicated components and transfering 48µl of the master mix to each reaction tube. Initiate the reaction by adding the template. Use individual pipette tips for all additions, being careful not to cross-contaminate the samples.

Component	Volume	Final Concentration
Nuclease-Free Water (to a final volume of 50µl)	Xµl	
AMV/*Tfl* 5X Reaction Buffer (see Note 1)	10µl	1X
dNTP Mix (10mM each dNTP; see Note 2)	1µl	0.2mM
downstream primer (see Note 3)	50pmol	1µM
upstream primer (see Note 3)	50pmol	1µM
25mM $MgSO_4$ (see Note 2)	2µl	1mM
AMV Reverse Transcriptase (5u/µl)	1µl	0.1u/µl
Tfl DNA Polymerase (5u/µl)	1µl	0.1u/µl
RNA sample or control (see Section 3.A and Note 4)	Yµl	
final volume	**50µl**	

2. Overlay the reaction with one or two drops (20–40µl) of nuclease-free mineral oil to prevent condensation and evaporation.
3. Place the tubes in a controlled temperature heat block equilibrated at 45°C and incubate for 45 minutes.
4. Proceed directly to thermal cycling the reactions for second strand cDNA synthesis and amplification (see the thermal cycling profile outlined above).

Notes:

1. If a precipitate forms in the AMV/*Tfl* 5X Reaction Buffer, resolubilize by incubating at 65°C for 15 minutes.
2. Vortex prior to use.
3. A general formula for calculating the number of nanograms of primer equivalent to 50pmol is: 50pmol = 16.3ng × b; where b is the number of bases in the primer. For the positive control reaction, use 3.3µl of both the Downstream and Upstream Control Primers (50pmol).
4. 10^3–10^6 copies of a specific target template or 1pg–1µg total RNA. Use 2µl of the Positive Control RNA with Carrier (2.5 attomoles or 1×10^6 copies).

4.B. Analysis

1. Analyze the PCR products by agarose gel electrophoresis of 5% of the total reaction. The products will be readily visible by UV transillumination of an ethidium bromide-stained gel. The amplification product obtained using the Positive Control RNA with the Upstream and Downstream Control Primers is 323bp long (Figure 4).
2. Store the reaction products at –20°C until needed. The reaction products may be purified using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281; 7).

   Note: An amplification product at approximately 220bp is occasionally observed with the Positive Control RNA. This product arises from the amplification of a sequence in the carrier *E. coli* RNA added to the Positive Control RNA.

```
     1    GAATACAAGC    TTGGGCGTGT    CTCAAAATCT    CTGATGTTAC    ATTGCACAAG
    51    ATAAAAATAT    ATCATCATGA    ACAATAAAAC    TGTCTGCTTA    CATAAACAGT
   101    AATACAAGGG    GTGTTATGAG    CCATATTCAA    CGGGAAACGT    CTTGCTCGAG
   151    GCCGCGATTA    AATTCCAACA    TGGATGCTGA    TTTATATGGG    TATAAATGGG
   201    CTCGCGATAA    TGTCGGGCAA    TCAGGTGCGA    CAATCTATCG    ATTGTATGGG
   251    AAGCCCGATG    CGCCAGAGTT    GTTTCTGAAA    CATGGCAAAG    GTAGCGTTGC
   301    CAATGATGTT    ACAGATGAGA    TGGTCAGACT    AAACTGGCTG    ACGGAATTTA
   351    TGCCTCTTCC    GACCATCAAG    CATTTTATCC    GTACTCCTGA    TGATGCATGG
   401    TTACTCACCA    CTGCGATCCC    CGGGAAAACA    GCATTCCAGG    TATTAGAAGA
   451    ATATCCTGAG    TCAGGTGAAA    ATATTGTTGA    TGCGCTGGCA    GTGTTCCTGC
   501    GCCGGTTGCA    TTCGATTCCT    GTTTGTAATT    GTCCTTTTAA    CAGCGATCGC
   551    GTATTTCGTC    TCGCTCAGGC    GCAATCACGA    ATGAATAACG    GTTTGGTTGA
   601    TGCGAGTGAT    TTTGATGACG    AGCGTAATGG    CTGGCCTGTT    GAACAAGTCT
   651    GGAAAGAAAT    GCATAAGCTT    TTGCCATTCT    CACCGGATTC    AGTCGTCACT
                                    ----------
          Upstream Control Primer   5´-GCCATTCT    CACCGGATTC    AGTCGTC-3´
   701    CATGGTGATT    TCTCACTTGA    TAACCTTATT    TTTGACGAGG    GGAAATTAAT
   751    AGGTTGTATT    GATGTTGGAC    GAGTCGGAAT    CGCAGACCGA    TACCAGGATC
   801    TTGCCATCCT    ATGGAACTGC    CTCGGTGAGT    TTTCTCCTTC    ATTACAGAAA
   851    CGGCTTTTTC    AAAAATATGG    TATTGATAAT    CCTGATATGA    ATAAATTGCA
   901    GTTTCATTTG    ATGCTCGATG    AGTTTTTCTA    ATCAGAATTG    GTTAATTGGT
   951    TGTAACACTG    GCAGAGCATT    ACGCTGACTT    GACGGGACGG    CGGCTTTGTT
                                    ----------
          Downstream Control Primer 3´-GACTGAA    CTGCCCTGCC      GCCGA-5´
  1001    GAATAAATCG    AACTTTTGCT    GAGTTGAAGG    ATCAGATCAC    GCATCTTCCC
  1051    GACAACGCAG    ACCGTTCCGT    GGCAAAGCAA    AAGTTCAAAA    TCACCAACTG
  1101    GTCCACCTAC    AACAAAGCTC    TCATCAACCG    TGGCGACTCT    AGAGGATCCC
  1151    CGGGCGAGCT    CCCAAAAAAA    AAAAAAAAAA    AAAAAAAAAA    AAACCGAATT
```

Figure 4. Sequence of the Positive Control RNA, Upstream Control Primer and Downstream Control Primer. The expected cDNA product is 323bp long.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Low yield or no first strand product	RNA degraded: • Verify the integrity of the RNA by denaturing agarose gel electrophoresis. • Ensure that reagents, tips and tubes are RNase-free. Isolate the RNA in the presence of a ribonuclease inhibitor (e.g., Recombinant RNasin® Ribonuclease Inhibitor).
	AMV Reverse Transcriptase thermally inactivated. If an initial denaturation/annealing step is introduced into the protocol, be certain to add the enzyme mix containing AMV Reverse Transcriptase **after** the denaturation step and subsequent 45°C equilibration.
	Primer specificity. Verify that the "downstream" primer was designed to be complementary to the downstream sequence of the RNA.
	Primer annealing. If oligo(dT) was used as a "downstream" primer, verify that the annealing incubation was carried out at an appropriate temperature, such as 37°C, prior to reverse transcription.
	RNA purification problem. Carryover of reagents (e.g., SDS, NaCl, heparin, guanidine thiocyanate) from some RNA purification methods can interfere with RT-PCR. Reduce volume of target RNA, perform additional purification steps or change purification method.
Amplification product has a molecular weight higher than expected	Genomic DNA sequences related to the RNA template contaminate the RNA preparation. Digest the DNA using RQ1 RNase-Free DNase.
Low yield or no amplification product	Insufficient number of cycles. Return reactions to thermal cycler for 5 more cycles.
	Thermal cycler programmed incorrectly. Verify that times and temperatures are correct.
	Temperature too low in some positions of thermal cycler. Perform a set of control reactions to determine if certain positions in the thermal cycler give low yields.
	Improper reaction conditions. Reduce the annealing temperature, and/or allow longer extension times for longer amplimers.
	Missing reaction component. Check the reaction components, and repeat the reaction.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Low yield or no amplification product (continued)	Mineral oil problem. The reaction must be overlaid with high-quality, nuclease-free light mineral oil. **Do not** use autoclaved mineral oil.
	Reaction tubes not autoclaved. Autoclaving tubes eliminates contaminants that inhibit amplification.
	Insufficient first strand product. See discussion above under "low yield or no first strand product".
	Poor primer design. Make sure primers are not self-complementary or complementary to each other. Check the length and T_m of the PCR primers.
	Incorrect primer specificity. Verify that the primers were designed to be complementary to the appropriate strands.
	Suboptimal reaction conditions. Optimize $MgSO_4$ concentration, annealing temperature and extension time. Verify that primers are present in equal concentration. Vortex the $MgSO_4$ prior to use.
	Nucleotides degraded. Keep nucleotides frozen in aliquots, thaw quickly and keep on ice once thawed. Avoid multiple freeze-thaw cycles.
	Target sequence not present in target RNA. Redesign experiment or try other sources of target RNA.
Multiple, nonspecific amplification products	Suboptimal reaction conditions. Optimize $MgSO_4$ concentration and annealing temperature. Vortex the $MgSO_4$ prior to use.
	Poor primer design. Make sure primers are not self-complementary or complementary to each other, especially near the 3´-ends. Check the length and T_m of the PCR primers. Avoid using three G or C nucleotides in a row at the 3´-end of a primer.
	Contamination by another target RNA/DNA. Use positive displacement pipets or aerosol-resistant tips to reduce cross contamination during pipetting. Use a separate work area and pipettor for pre- and post-amplification. Wear gloves and change them often. Use UNG (4) or another sterilization technique to prevent DNA carryover to subsequent reactions.
	Multiple target sequences exist in the target RNA. Design new primers.

6. References

1. Miller, K. and Storts, D. (1995) PCR Access! A sensitive single-tube two-enzyme system for RT-PCR. *Promega Notes* **53**, 2–5.
2. Kaledin, A.S., Slyusarenko, A.G. and Gorodetskii, S.I. (1981) Isolation and properties of DNA polymerase from the extreme thermophilic bacterium *Thermus ruber. Biokhimiia* **46**, 1576–84.
3. Blumberg, D.D. (1987) Creating a ribonuclease-free environment. *Meth. Enzymol.* **152**, 20–4.
4. Longo, M.C., Berninger, M.S. and Hartley, J.L. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* **93**, 125–8.
5. Sellner, L.N., Coelen, R.J. and Mackenzie, J.S. (1992) Reverse transcriptase inhibits *Taq* polymerase activity. *Nucl. Acids Res.* **20**, 1487–90.
6. Chumakov, K.M. (1994) Reverse transcriptase can inhibit PCR and stimulate primer-dimer formation. *PCR Meth. Appl.* **4**, 62–4.
7. *Wizard® SV Gel and PCR Clean-Up System Technical Bulletin* #TB308, Promega Corporation.

7. Appendix

7.A. Composition of Buffers and Solutions

dNTP Mixture, 10mM

10mM	each of dATP, dCTP, dGTP and dTTP in water

Positive Control RNA with Carrier

1.25amol/µl	1.2kb kanamycin resistance gene mRNA (prepared by in vitro transcription)
3µg/ml	*E. coli* tRNA (carrier)
10mM	Tris-HCl (pH 8.0)
0.1mM	EDTA

$MgSO_4$ Solution

25mM	$MgSO_4$ in water

TAE 50X buffer

242g	Tris base
57.1ml	glacial acetic acid
100ml	0.5M EDTA (pH 8.0)

Add deionized water to 1 liter.

7.B. Control Primer Sequences

Upstream Control Primer: 5´GCC ATT CTC ACC GGA TTC AGT CGT C 3´

Downstream Control Primer: 5´AGC CGC CGT CCC GTC AAG TCA G 3´

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7.C. Related Products

Amplification

Product	Size	Cat.#
GoTaq® Green Master Mix	100 reactions	M7122
	1,000 reactions	M7123

GoTaq® Green Master Mix is a premixed solution of GoTaq® DNA Polymerase, GoTaq® Green Reaction Buffer, dNTPs and Mg^{2+}.

Product	Conc.	Size	Cat.#
GoTaq® DNA Polymerase	5u/µl	100u	M3001

Available in additional sizes.

Product	Conc.	Size	Cat.#
GoTaq® Flexi DNA Polymerase	5u/µl	100u	M8291

GoTaq® Flexi DNA Polymerase contains 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and Magnesium Chloride Solution, 25mM. Reaction buffers are magnesium-free. Available in additional sizes.

Product	Size	Cat.#
AccessQuick™ RT-PCR System	20 reactions	A1701

Available in additional sizes.

Reverse Transcription

Product	Conc.	Size	Cat.#
AMV Reverse Transcriptase	10u/µl	300u*	M5101
M-MLV Reverse Transcriptase	200u/µl	10,000u*	M1701
M-MLV Reverse Transcriptase, RNase H Minus	100–200u/µl	10,000u	M5301
Reverse Transcription System		100 reactions	A3500
ImProm-II™ Reverse Transcriptase		100 reactions*	A3802

*Available in additional sizes.

RNA Purification

Product	Size	Cat.#
PureYield™ RNA Midipreps System	10 preps	Z3740
SV Total RNA Isolation System	50 preps	Z3100

Available in additional sizes.

PCR Cloning

Product	Size	Cat.#
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II	20 reactions	A1380
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610
pTarget™ Mammalian Expression Vector System	20 reactions	A1410

The pGEM®-T Vector System II, pGEM®-T Easy Vector System II and pTarget™ Mammalian Expression Vector System include JM109 Competent Cells (High Efficiency).

PCR Fragment Purification

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281

Available in additional sizes.

Other Related Products

Product	Conc.	Size	Cat.#
Recombinant RNasin® Ribonuclease Inhibitor	20–40u/µl	2,500u*	N2511
RNasin® Ribonuclease Inhibitor	20–40u/µl	2,500u*	N2111
RQ1 RNase-Free DNase	1u/µl	1,000u	M6101
100bp DNA Step Ladder	1µg/µl	100µg	G6951
PCR Nucleotide Mix	10mM each	200µl*	C1141
dATP, dCTP, dGTP, dTTP	100mM each	40µmol each	U1240
dUTP, dATP, dCTP, dGTP	100mM each	40µmol each	U1245

*Available in additional sizes.

8. Summary of Changes

The following changes were made to the 7/24 revision of this document:

1. In Section 7.A, under Positive Control RNA with Carrier, rRNA was updated to tRNA.
2. Storage temperatures in Section 2 were updated to reflect new temperature ranges, without changing the actual storage temperatures.
3. Patent and disclaimer statements were updated.
4. The cover image and font were updated and miscellaneous minor text edits made.
5. Related Products, Section 7.C, was updated, including removal of discontinued products.

[a]RT-PCR reactions at temperatures above 45°C are covered by U.S. Pat. Nos. 5,817,465 and 5,654,143 and European Pat. No. 0 568 272.