

Quicker Access to Results

AccessQuick™ RT-PCR System: Simple, Stable and Sensitive

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Abstract

Promega's new AccessQuick™ RT-PCR System provides a convenient, one-step method for coupled RT-PCR. The AccessQuick™ Master Mix, a stable 2X solution, contains all necessary reaction components except primers, template RNA and AMV-RT, reducing setup time and pipetting steps. In testing diverse target RNAs, we found that the AccessQuick™ RT-PCR System provides the sensitivity and yield that researchers have today in the Access RT-PCR System.

The simplified reaction offers greater convenience by saving time and decreasing the possibility of contamination and errors because of fewer pipetting steps.

Introduction

One-step, coupled RT-PCR is a single-tube reaction in which a reverse transcriptase produces first strand cDNA from either total RNA or mRNA, then a thermostable DNA polymerase produces second strand DNA and amplifies the specific DNA of interest. In this technique, target-specific primers can be used to determine the presence of a transcript, to quantitate expression levels or to clone cDNA products.

Building upon the popularity of the Access RT-PCR System^(a,b), Promega is introducing the AccessQuick™ RT-PCR System^(a,b) to further simplify RT-PCR. In this new system we combine buffer, magnesium, dNTPs, and *Tfl* DNA Polymerase^(a) into the 2X AccessQuick™ Master Mix. To perform RT-PCR, just add RNA template, target-specific primers, AMV Reverse Transcriptase (AMV-RT) and Nuclease-Free Water to the Master Mix. AMV reverse transcriptase is provided because in our buffer it has optimal activity at a higher temperature than M-MLV RT (48°C versus 37°C). This makes it useful for reverse transcription of RNA that has secondary structure (1).

The simplified reaction offers greater convenience by saving time and decreasing the possibility of contamination and errors because of fewer pipetting steps. Here we demonstrate the sensitivity and stability of the

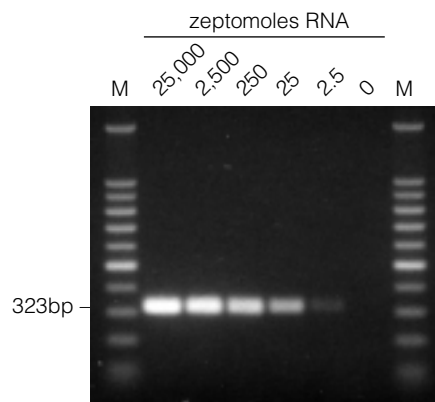


Figure 1. Detection sensitivity of the AccessQuick™ RT-PCR System. A 323bp RT-PCR product complementary to the kanamycin resistance gene was amplified using gene-specific primers [2] and the indicated amounts of RNA (1.2kb Kanamycin Positive Control RNA^(b); Cat.# C1381). RT-PCR was performed according to the AccessQuick™ System protocol (3). RT-PCR profile: 48°C for 45 minutes, 94°C for 2 minutes, 30 cycles (94°C for 30 seconds, 60°C for 1 minute, 68°C for 2 minutes), 68°C for 5 minutes. To analyze the reactions, 8µl of each reaction were subjected to electrophoresis on a 1% agarose gel and DNA was detected by ethidium bromide staining. Lane M, 100bp DNA Ladder (Cat.# G2101).

AccessQuick™ RT-PCR System. We also compare detection sensitivity of this new system to the Access RT-PCR System using several targets, including RNAs of different relative abundance from several sources.

Sensitivity

When only a small amount of RNA is available, or when the RNA of interest is of low abundance, the sensitivity of an RT-PCR system is very important. To demonstrate sensitivity of the AccessQuick™ RT-PCR System, we used the Promega 1.2kb Kanamycin Positive Control RNA^(b), a polyadenylated RNA transcript, as a template. We amplified a 323bp RT-PCR product from serial dilutions of the RNA using gene-specific primers (2). As seen in Figure 1, the AccessQuick™ RT-PCR System demonstrated excellent sensitivity, amplifying the fragment and producing a discernable band with only 2.5 zeptomoles (2.5×10^{-21} moles) of RNA template.

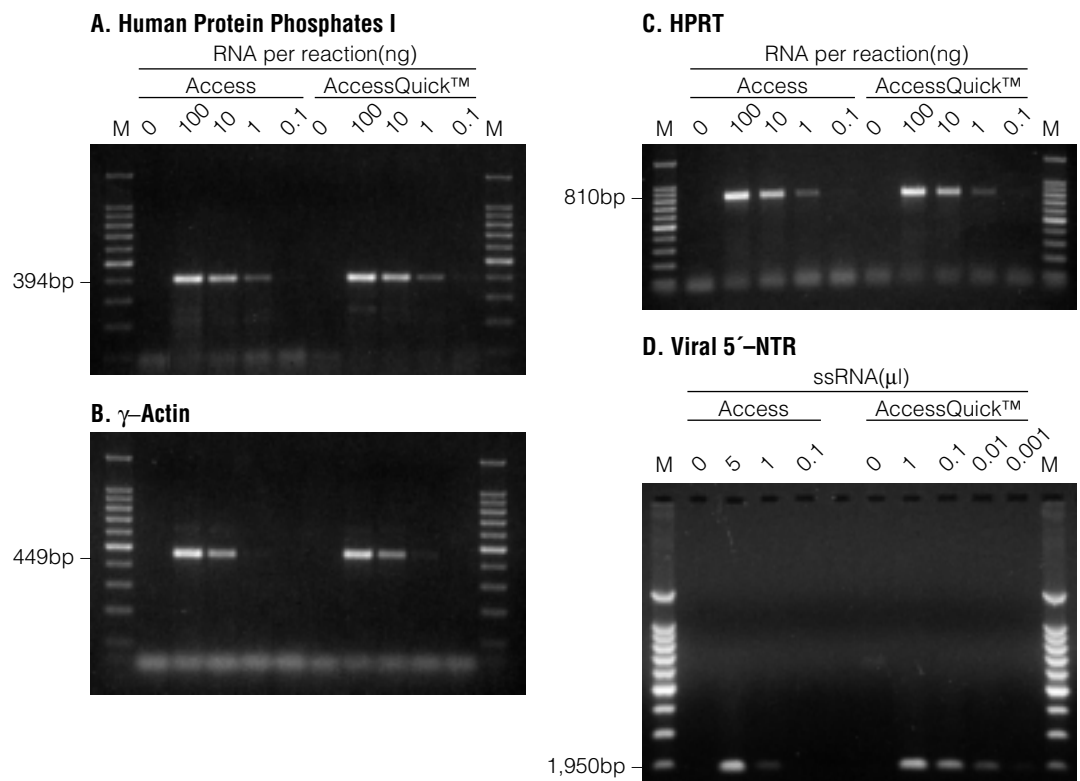


Figure 2. Comparison of Access and AccessQuick™ RT-PCR Systems using different RNA sources and target-specific primers. **Panel A:** Human Protein Phosphatase I mRNA product (394bp). **Panel B:** Human γ -actin mRNA product (449bp; 4,5). **Panel C:** Human hypoxanthine phosphoribosyl transferase (HRPT) mRNA product (810bp). **Panel D:** 5'-NTR ssRNA virus product (195bp; 6). RT-PCR was performed according to the recommended protocols (2,3). Template RNAs used included human Jurkat total RNA (**Panels A, B and C**), and viral ssRNA containing 5' NTR (**Panel D**). Reactions were analyzed as in Figure 1 on a 1 or 2% agarose gel. Lanes M, 100bp DNA Ladder.

Detection Sensitivity

We compared detection sensitivity and yield of Promega's Access and AccessQuick™ RT-PCR Systems using diverse targets from total RNA of both cultured human Jurkat and K562 cells (data not shown) as well as a ssRNA virus. Target transcripts varied in size (195 to 810bp) and messages varied in relative abundance (low to high).

We found the AccessQuick™ RT-PCR System produced results equivalent to or better than those obtained with the Access RT-PCR System for all targets tested (Figure 2). The yield and sensitivity of both systems were comparable for most mRNA targets. However, with a viral 5'-NTR target, the AccessQuick™ RT-PCR System resulted in significantly improved yield and sensitivity (>100-fold) compared to the Access RT-PCR System (Figure 2, Panel D).

We included targets of different relative abundance in these experiments (Figure 2). The human β -actin mRNA has a

high relative abundance (0.5–1%; 4,7) (data not shown), human γ -actin mRNA has a medium relative abundance (0.3–0.46%; 4,5), and human protein phosphatase 1 mRNA has low relative abundance (0.02%, 5). With all four products, the AccessQuick™ RT-PCR System demonstrated sensitivities that were comparable to those obtained with the Access RT-PCR System.

Stability

We evaluated the AccessQuick™ RT-PCR System for stability by repeatedly freezing and thawing the AccessQuick™ RT-PCR Master Mix up to ten times before use. We conducted two sets of freeze-thaw experiments: the first consisted of slow cycling from -20°C to 25°C , and the second consisted of rapid cycling from -70°C to 50°C . After five cycles, and again after ten cycles, we added AMV-RT, primers and RNA and performed RT-PCR to amplify a 323bp product. As evident in Figure 3, there is no noticeable drop in the band intensity after ten freeze-thaw cycles.

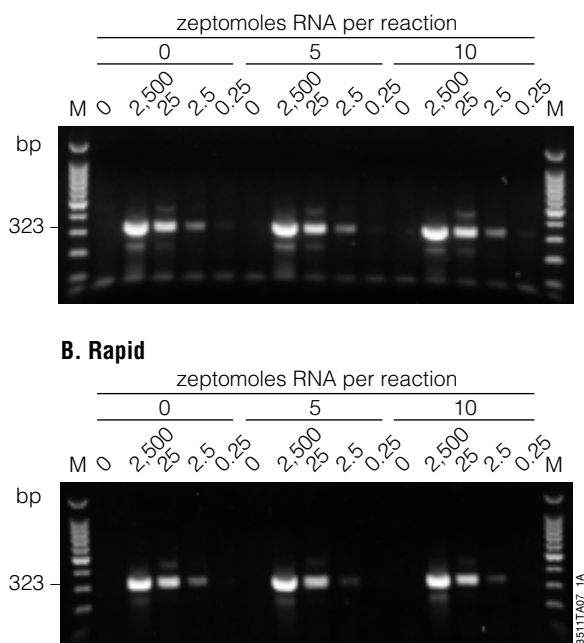


Figure 3. Stability of AccessQuick™ Master Mix through multiple freeze-thaw events. Both slow (Panel A) and rapid (Panel B) freeze-thaw events were performed by allowing the AccessQuick™ RT-PCR System reaction mix (Cat.# A1701) to thaw from -20°C to room temperature (slow) or from -70°C to 50°C (rapid). A 323bp RT-PCR product (2) was amplified from 1.2kb Kanamycin Positive Control RNA after 0, 5 and 10 freeze-thaw cycles using the indicated amounts of RNA. Reactions were incubated and analyzed as in Figure 1, except that 10µl of each reaction was loaded on a 2% agarose gel. Lanes M, 100bp DNA Ladder.

Conclusions

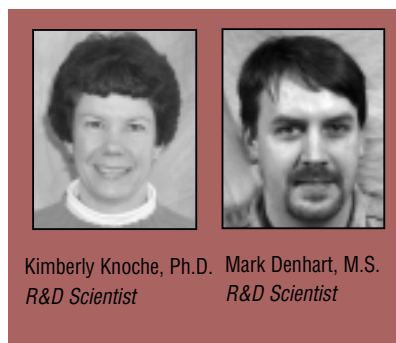
The AccessQuick™ RT-PCR System is a one-step, coupled RT-PCR system that works as well as the Access RT-PCR System. All major reaction components are incorporated into a Master Mix, which greatly simplifies and accelerates sample processing. The AccessQuick™ RT-PCR System has excellent sensitivity (to the zeptomole range in some cases), and the Master Mix is stable to at least 10 freeze-thaw cycles.

References

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2. *Access RT-PCR System and Access RT-PCR Introductory System Technical Bulletin #TB220*, Promega Corporation.
3. *AccessQuick™ RT-PCR System Promega Product Information #9PIA170*, Promega Corporation.
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7. Scheuermann, R.H. and Bauer, S.R. (1993) *Meth. Enzymol.* **218**, 446–473.

Protocols

- ◆ *AccessQuick™ RT-PCR System Promega Product Information #9PIA170*, Promega Corporation. (www.promega.com/tbs/9pia1703/9pia170.html)
- ◆ *Access RT-PCR System and Access RT-PCR Introductory System Technical Bulletin #TB220*, Promega Corporation. (www.promega.com/tbs/tb220/tb220.html)



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Ordering Information

Product	Size	Cat.#
AccessQuick™ RT-PCR System	500 reactions	A1703
	100 reactions	A1702
	20 reactions	A1701
	10 reactions	A1700
Access RT-PCR System	500 reactions	A1280
	100 reactions	A1250
Access RT-PCR Introductory System	20 reactions	A1260
100bp DNA Ladder	250µl	G2101
1.2kb Kanamycin Positive Control RNA	5µg	C1381

AccessQuick is a trademark of Promega Corporation.

^(a)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

^(b)U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

Technical Questions?

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