Certificate of Analysis

AccessQuick™ RT-PCR System

Part# 9PIA170

Revised 4/18

Cat.#	Size
A1700	10 reactions
A1701	20 reactions
A1702	100 reactions
A1703	500 reactions

Description: AccessQuick™ RT-PCR System[a–c] is provided in quantities sufficient for 10–500 one-tube RT-PCR reactions of 50µl each. **Primers and templates are required to perform the reactions.** Contains one of the following:

AF9PIA170 0418A170

Cat.# A1700

Part No.	Component	Size
A170A	AccessQuick™ Master Mix (2X)	250µl
A261B	AMV Reverse Transcriptase	100u (5u/µl)
P119A	Nuclease-Free Water	1,250µl

Cat.# A1701

Part No.	Component	Size
A170A	AccessQuick™ Master Mix (2X)	2 × 250µl
A261B	AMV Reverse Transcriptase	100u (5u/µl)
P119A	Nuclease-Free Water	1,250µl

Cat.# A1702

Part No.	Component	Size
A170B	AccessQuick™ Master Mix (2X)	3 × 1ml
A261A	AMV Reverse Transcriptase	500u (5u/µl)
P119A	Nuclease-Free Water	2 x 1,250µl

Cat.# A1703

Part No.	Component	Size
A170C	AccessQuick™ Master Mix (2X)	12.5ml
A261C	AMV Reverse Transcriptase	2,500u (5u/µl)
P119C	Nuclease-Free Water	25ml

Storage Conditions: Store the AccessQuick™ RT-PCR System at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

Quality Control Assays

This batch of product meets the following quality control release testing criteria:

Functional Testing

These reagents have been tested in RT-PCR. 2.5 zeptomoles (approximately 1,000 copies) of template RNA, 1.2kb Kanamycin Positive Control RNA (Cat.# C1381), was amplified using the Upstream and Downstream Control Primers supplied in the Access RT-PCR System (Cat.# A1250). The resulting 323bp DNA product was detected by ethidium bromide staining following agarose gel electrophoresis.

Nuclease Contamination

The components of this system are tested for RNase and DNase contamination using a tritiated substrate assay. No contaminating RNase or DNase activities were detected.

[a]Use of this product for basic PCR is outside of any valid US or European patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research, commercial or diagnostic applications without any license or royalty fees.
[b]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.
[c]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIA170
Printed in USA. Revised 4/18.

Usage Information

I. Standard RT-PCR Protocol

The AccessQuick™ RT-PCR System is an easy and convenient master mix system for setting up one-tube RT-PCR reactions. It is designed to increase the convenience of performing RT-PCR by combining the following components in a single tube:
Tfl DNA Polymerase, dNTPs, magnesium sulfate and reaction buffer. The AMV RT enzyme is provided in a separate tube for the important, no-RT control reaction. The AccessQuick™ Master Mix is simply added to RNA templates in reaction vials, followed by the AMV RT and primers.

A. Prepare Reactions

1. Use sterile, nuclease-free tubes.
2. For each 50µl reverse transcription (RT) reaction, combine:

Component	Volume	Final Conc.
AccessQuick™ Master Mix, 2X	25µl	1X
Upstream Primer, 10µM	0.5–5.0µl	0.1–1.0µM
Downstream Primer, 10µM	0.5–5.0µl	0.1–1.0µM
RNA Template	1–5µl	1pg-1µg
Nuclease-Free Water to a final volume of	50µl	

Ensure that the AccessQuick™ Master Mix is thoroughly mixed before removing aliquots.

3. Add 1µl (5u) AMV Reverse Transcriptase as the final component and mix by gentle vortexing or pipetting.

Notes:

- If working with multiple samples, a master mix may be assembled on ice by combining appropriate multiples of each of the indicated components and transferring an aliquot 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.
- For assistance in calculating melting temperatures of oligonucleotides in the AccessQuick™ Reaction Buffer, please use the T_m Calculator at: www.promega.com/biomath/

B. Reverse Transcription

1. Incubate the reaction tubes at 45°C for 45 minutes.
2. Proceed with PCR cycling.

Note:

- Conditions may require optimization. We recommend 45°C for 45 minutes as a starting point; however, efficient first-strand cDNA synthesis can be accomplished in a 15- to 60-minute incubation at 37–45°C.

C. PCR Amplification

Denaturation

Generally, a 2-minute initial denaturation step at 95°C is sufficient. Subsequent denaturation steps will be between 30 seconds and 1 minute.

Annealing

Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.

The annealing step is typically 30 seconds to 1 minute at 55–65°C.

Extension

The extension reaction is typically performed at the optimal temperature for *Tfl* DNA Polymerase, which is 68–72°C. Allow 30 seconds to 1 minute for every 1kb of DNA to be amplified. When targeting small amplicons (<500bp) using reasonable levels
of template, this extension step may be unnecessary.

A final extension of 5 minutes at 68–72°C is recommended.

Soak Cycle

Hold the reactions at 4°C overnight. Long-term storage at –20°C is recommended.

Cycle Number

Generally, 25–30 cycles result in optimal amplification of desired products. Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

Part# 9PIA170
Printed in USA. Revised 4/18