Certificate of Analysis

AccessQuick™ RT-PCR System

Part# 9PIA170
Revised 7/25

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

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

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 × 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

Expiration Date: See product label for expiration date.

Storage Conditions: See the product label for storage temperature recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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.

Signed by:

R. Wheeler, Quality Assurance

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Promega

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Usage Information

1. 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.

1.A. Prepare Reactions

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

Reaction Buffer	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/**

1.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.

1.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.

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