# GeneAmp® RNA PCR Core Kit

## Package Contents

| Catalog Number | Size |
|---|---|
| N8080143 | 100 rxns |

Kit Contents

## Storage Conditions

- Store all contents at –20°C until just prior to use.
- Reverse Transcriptase and RNase Inhibitor are sensitive to air oxidation.

## Required Materials

- Template: RNA
- Forward and reverse gene-specific primers
- DEPC-treated water (Cat. no. AM9916)
- E-Gel® General Purpose Gels, 1.2% (Cat. no. G5018-01)
- TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- *Optional*: 1 M stock DTT (Cat. no. P2325)

## Timing

Varies depending on amplicon length

## Selection Guides

Go online to view related products.
PCR Enzymes and Master Mixes

## Product Description

- The GeneAmp® RNA PCR Core Kit is designed for use in detection and analysis of RNA gene expression.
- Murine Leukemia Virus (MuLV) Reverse Transcriptase is included for reverse transcription of RNA to cDNA and AmpliTaq® DNA Polymerase for subsequent PCR amplification.
- A recombinant RNase Inhibitor is included for inhibition of certain mammalian RNases.

## Important Guidelines

- Select the correct polymerase, PCR instrument, and cycling conditions for your application.
- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- Dilute the 1 M DTT stock to 100 mM final in water prior to use in your RT reaction.

## Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

**For Research Use Only. Not for use in diagnostic procedures.**

## RT Characteristics: MuLV

## Polymerase Characteristics

| | |
|---|---|
| **Hot-start:** | N/A |
| **Length:** | Up to 5 kb |
| **Fidelity vs. *Taq*:** | 1X |
| **Format:** | Separate components |

## RT Reaction Setup

Use the measurements below to prepare your RT experiment, or enter your own parameters in the column provided.

| Component | 20-µL rxn | Custom | Final Conc. |
|---|---|---|---|
| DEPC-treated water | to 20 µL | to µL | — |
| 10X PCR Buffer II | 2 µL | µL | 1X |
| 25 mM $MgCl_2$ | 4 µL | µL | 5 mM |
| 10 mM dATP | 2 µL | µL | 1 µM |
| 10 mM dCTP | 2 µL | µL | 1 µM |
| 10 mM dGTP | 2 µL | µL | 1 µM |
| 10 mM dTTP* | 2 µL | µL | 1 µM |
| 100 mM DTT (optional)** | 1 µL | µL | 5 mM |
| 50 µM Oligo $d(T)_{16}$,<br>50 µM Random hexamers, or<br>10 µM Gene-specific reverse primer | 1 µL<br>1 µL<br>1 µL | µL | 2.5 µM<br>2.5 µM<br>0.5 µM |
| Template RNA | varies | µL | < 1 µg/rxn |
| RNase Inhibitor (20 U/µL) | 1 µL | µL | 1 U/µL |
| MuLV RT (50 U/µL) | 1 µL | µL | 2.5 U/µL |

* dUTP can be substituted for dTTP for UNG (Cat. no. N8080096).
** Dilute the 1 M DTT stock to 100 mM final in water prior to use in your RT reaction.
For qPCR instructions, refer to AmpErase® Uracil N-Glycosylase (UNG) MAN0009788.

## RT, PCR, and One-Step RT-PCR Protocols

See pages 2, 3, and 4 to view procedures for preparing and running your RT, PCR, or One-Step RT-PCR experiments.

## Optimization Strategies

Refer to the pop-ups below for guidelines to optimize your RNA Samples and RT or PCR reactions.

**RNA Sample Prep** **RT Guidelines** **PCR Guidelines**

**Limited Warranty, Disclaimer, and Licensing Information**

Print Options

# Reverse Transcription (RT) Protocol

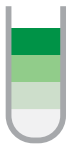
The example procedure below shows reagent volumes for a single **20-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube prior to adding sample and primers.

## 1 Thaw reagents

Thaw, mix, and briefly centrifuge each component before use. Set up the reactions on ice.

**Note:** Avoid generating bubbles when mixing the enzyme.

## 2 Prepare reverse transcription master mix



Add the following components to each reaction tube.

**Note:** Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.

| Component | 20-µL rxn | Final Concentration |
|---|---|---|
| DEPC-treated water | to 20 µL* | — |
| 10X PCR Buffer II | 2 µL | 1X |
| 25 mM $MgCl_2$ | 4 µL | 5 mM |
| 10 mM dNTPs (each) | 2 µL (each) | 1 µM (each) |
| 100 mM DTT (optional) | 1 µL | 5 mM |
| RNase Inhibitor (20 U/µL) | 1 µL | 1 U/µL |
| MuLV Reverse Transcriptase (50 U/µL) | 1 µL | 2.5 U/µL |

* Any combination of water and template RNA volumes can be used a long as the final volume is 20 µL.

Cap, mix, and briefly centrifuge the components.

## 3 Add template RNA and primers

Add your primers and sample to each tube for a final reaction volume of 20 µL.

**Note:** If using oligo $d(T)_{16}$ or reverse gene specific primers, incubate RNA and primer following the first two cycling steps (highlighted below), then add master mix and continue with the next three cycling steps. For random hexamers, combine the master mix, primer, and RNA, and then cycle as noted below.

| Component | 20-µL rxn | Final Concentration |
|---|---|---|
| 50 µM Oligo $d(T)_{16}$,<br>50 µM Random hexamers, or<br>10 µM Gene-specific reverse primer | 1 µL<br>1 µL<br>1 µL | 2.5 µM<br>2.5 µM<br>0.5 µM |
| Template RNA | varies | ≤ 1 µg total RNA/20-µL rxn |

Cap each tube, mix, and then briefly centrifuge the contents.

## 4 Incubate reactions in a thermal cycler

| Primer Type | Temperature | Time |
|---|---|---|
| Oligo $d(T)_{16}$ or gene-specific primers | 65°C | 5 minutes |
| | 4°C | 2 minutes |
| | 37°C | 30 minutes |
| | 95°C | 5 minutes |
| | 4°C | indefinitely |
| Random hexamers | 25°C | 10 minutes |
| | 37°C | 30 minutes |
| | 95°C | 5 minutes |
| | 4°C | indefinitely |

# PCR Amplification Protocol

The example PCR procedure below shows reagent volumes for a single **100-µL** reaction, including the 20-µL reverse transcription (RT) reaction mix described in the RT Protocol (page 2). For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense the appropriate volumes into each reverse transcription reaction tube prior to adding the primers.

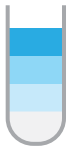
## 1 Thaw reagents

Thaw, mix, and briefly centrifuge each component before use.

**Note:** Avoid generating bubbles when mixing the enzyme.

**Note:** Consider the volumes for all components listed in steps 2–4 to determine the correct amount of water required to reach your final reaction volume.

## 2 Prepare PCR master mix

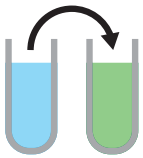


Combine the following components in each reaction tube.

| Component | 100-µL rxn | Final Concentration |
|---|---|---|
| Autoclaved, distilled water | to 76 µL | — |
| 10X PCR Buffer II | 8 µL | 1X |
| 25 mM $MgCl_2$ | 8 µL | 2 mM |
| AmpliTaq® DNA Polymerase (5 U/µL) | 0.5 µL | 0.025 U/µL |

Mix and briefly centrifuge the components.

## 3 Add PCR master mix to reverse transcription reaction tubes



Dispense 76 µL of the PCR master mix into each 20-µL reverse transcription reaction tube.

| Component | 100-µL rxn |
|---|---|
| PCR master mix (from step 2) | 76 µL |
| RT reaction mix (from RT protocol) | 20 µL |

## 4 Add primers

Add your primers to each tube for a final reaction volume of **100 µL**.

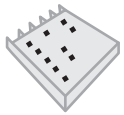
| Component | 100-µL rxn | Final Concentration |
|---|---|---|
| 10 µM forward primer | 2 µL | 0.2 µM |
| 10 µM reverse primer | 2 µL | 0.2 µM |
| **Total** | **100 µL** | |

Cap, mix, and then centrifuge the contents.

## 5 Incubate reactions in a thermal cycler

| | Step | Temperature | Time |
|---|---|---|---|
| 1 cycle | Initial denaturation | 95°C | 2 minutes |
| 35 PCR cycles | Denature | 95°C | 15 seconds |
| | Anneal | ~55°C (depending on primer $T_m$) | 30 seconds |
| | Extend | 72°C | 1 minute/kb |
| 1 cycle | Final extension | 72°C | 7 minutes |
| Hold | | 4°C | indefinitely |

## 6 Analyze with gel electrophoresis



Analyze 10 µL using agarose gel electrophoresis.

Use your PCR reaction immediately for down-stream applications, or store it at –20°C.

# One-Step (endpoint) RT-PCR Protocol

The example procedure below shows reagent volumes for a single **20-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube prior to adding sample and primers.

## 1 Thaw reagents

Thaw, mix, and briefly centrifuge each component before use.

**Note:** Avoid generating bubbles when mixing the enzyme.

**Note:** Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.

## 2 Prepare PCR master mix

Combine the following components in each reaction tube.

| Component | 20-µL rxn | Final Concentration |
|---|---|---|
| DEPC-treated water | 14.85 µL | — |
| 10X PCR Buffer II | 2.0 µL | 1X |
| 25 mM $MgCl_2$ | 1.4 µL | 1.75 mM |
| 10 mM dATP | 0.4 µL | 0.2 µM |
| 10 mM dCTP | 0.4 µL | 0.2 µM |
| 10 mM dGTP | 0.4 µL | 0.2 µM |
| 10 mM dTTP | 0.4 µL | 0.2 µM |
| 100 mM DTT (optional) | 1.0 µL | 5 mM |
| RNase Inhibitor (20 U/µL) | 0.2 µL | 0.2 U/µL |
| MuLV Reverse Transcriptase (50 U/µL) | 0.12 µL | 0.3 U/µL |
| AmpliTaq® DNA Polymerase (5 U/µL) | 0.2 µL | 0.05 U/µL |

Mix and briefly centrifuge the components.

## 3 Add primers and template RNA

Add your primers and template RNA to each reaction tube.

| Component | 20-µL rxn | Final Concentration |
|---|---|---|
| 10 µM forward gene-specific primer | 0.8 µL | 0.4 µM |
| 10 µM reverse gene-specific primer | 0.8 µL | 0.4 µM |
| Template RNA | varies | < 1 µg |

Cap, mix, and then centrifuge the contents.

## 4 Incubate reactions in a thermal cycler

| Step | Temperature | Time |
|---|---|---|
| RT step | 37°C | 30 minutes |
| Initial denaturation | 95°C | 2 minutes |
| 35 PCR cycles | 95°C | 20 seconds |
| | 60°C | 1 minute/kb |
| Final extension | 72°C | 7 minutes |
| Hold | 4°C | indefinitely |

## 5 Analyze with gel electrophoresis

Analyze 10 µL using agarose gel electrophoresis.

Use your PCR reaction immediately for down-stream applications, or store it at –20°C.