USER GUIDE

applied biosystems
life technologies

AgPath-ID™ One-Step RT-PCR Reagents

Core reagents for one-step qRT-PCR detection of pathogen

Catalog Numbers AM1005, 4387424, 4387391

Publication Number 1005M
Revision Rev. H

For Veterinary Use Only.

life technologies

The information in this guide is subject to change without notice.

DISCLAIMERS

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

LIMITED USE LABEL LICENSE No. 460: PCR Veterinary Diagnostics

Notice to Purchaser: For veterinary diagnostic services including reporting the results of such services for a fee and for research purposes only. Human diagnostic uses require a separate license from Roche.

TRADEMARKS

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TRI Reagent is a registered trademark of Molecular Research Center, Inc. AmpliTaq Gold® and TaqMan® are registered trademarks of Roche Molecular Systems, Inc, used under permission and license. Stratagene and MX300P are registered trademarks of Agilent Technologies, Inc. SmartCycler is a registered trademark of Cepheid Corporation.

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description
H	March, 2015	Clarified that the kit is intended for use with single- or duplex assays. Other format, style, and legal updates
G	October 2012	Baseline for this revision history

Product information

Purpose of the product

The AgPath-ID™ One-Step RT-PCR Reagents are designed for sensitive, robust amplification of RNA targets using a single-tube TaqMan® real-time reverse transcription PCR (RT-PCR) strategy. The kit is optimized for use with single-plex or duplex TaqMan® primer/probe sets, and it includes:

- 25X RT-PCR Enzyme Mix: containing ArrayScript™ Reverse Transcriptase and AmpliTaq Gold® DNA Polymerase
- 2X RT-PCR Buffer: includes ROX™ passive reference dye for quantitative fluorescent signal normalizaton
- Detection Enhancer: an optional component for RT-PCR that may improve amplification of templates with high GC content or persistent secondary structure

For higher order multiplexed assays or samples that have been extracted from matrices with high inhibitor content (e.g., fecal samples and oral fluids), use the Path-ID™ Multiplex One-Step RT-PCR Kit (Cat. no. 4442136).

Reagents provided and storage conditions

Component	Cat. no. AM1005 100 rxns	Cat. no. 4387424 500 rxns	Cat. no. 4387391 1000 rxns
2X RT-PCR Buffer	1375 µL	7 mL	14 mL
25X RT-PCR Enzyme Mix	110 µL	550 µL	1100 µL
Detection Enhancer	190 µL	1.2 mL	2 x 1.2 mL
Nuclease-free Water	1.75 µL	25 mL	25 mL

- Store the AgPath-ID™ One-Step RT-PCR Reagents in a –10°C to –30°C non-frost-free freezer.
- Nuclease-free Water may be stored at –10°C to –30°C, 2°C to 8°C, or at room temperature.

Other required material

RNA sample(s)

Use pure RNA that is free of RT-PCR inhibitors in the procedure. We recommend using the MagMAX™ RNA Isolation Kit appropriate for your sample type. Go to **www.lifetechnologies.com**, then search for **MagMAX**.

When isolating viral RNA from cell-free sample sources such as serum, use MagMAX™ Viral RNA Isolation Kits that include carrier RNA to maximize RNA recovery.

PCR primer/ TaqMan® probe mixture

Single- and duplex TaqMan® primer/probe sets that are compatible with your real-time PCR instrument and designed for one-step RT-PCR can be used with the kit. You may need to optimize the concentration of primers and probe, but the concentrations shown in Table 1 typically work well.

Note: The Reverse Transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final mastermix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences not contained in the expression vector.

Table 1 Recommended PCR Primer/TaqMan® Probe Concentrations

Component	Final concentration in the reaction	25X primer/probe mix†
Forward PCR primer	400 nM	10 µM
Reverse PCR primer	400 nM	10 µM
TaqMan® probe	120 nM	3 µM

† Use 1 µL per 25-µL RT-PCR of a PCR primer/TaqMan® probe mixture prepared at these concentrations.

Plasticware

Plasticware includes:

- 96-well plates or tubes appropriate for real-time PCR
- Nuclease-free pipettors and tips, reagent reservoirs or tubes for preparing master mixes

Thermal cycler capable of real-time detection

Performance of the kit has been verified on the following systems:

- ABI Prism 7500 Sequence Detection System, Applied Biosystems® 7500 Real-Time PCR System, and Applied Biosystems® 7900HT Fast Real-Time PCR System
- Stratagene® Mx3000P® System
- Cepheid SmartCycler® II System

Methods

Program the real-time PCR instrument

Use the thermal cycling conditions shown in the following table.

- ROX™ passive reference dye is included in the RT-PCR Buffer.
- Reaction volume is 25 µL.

Step	Stage	Reps	96-well machines‡		SmartCycler II	
			Temp	Time	Temp	Time
Reverse transcription	1	1	45°C	10 min	45°C§	10 min
RT inactiv./initial denaturation	2	1	95°C	10 min	95°C	15 min
Amplification	3	40	95°C	15 sec	95°C	15 sec
Set ramp rates to 1.6°C/sec for SmartCycler†			60°C	45 sec	60°C	60 sec

† It is critically important to set the ramp rates (heating and cooling) to 1.6°C/sec for SmartCycler II reactions, otherwise amplification may fail.

‡ Settings for Applied Biosystems® 7500 and 7900HT, and Stratagene Mx3000P.

§ 50°C may be a more effective RT temperature for some PCR primer sets.

Assemble RT-PCRs

Follow the instructions to assemble RT-PCRs:

1. Prepare RT-PCR master mix(es) on ice (25 µL final).
   - Prepare 5–10% extra master mix.
   - Negative controls: Include duplicate no-template controls using nuclease-free water in place of sample.
2. Distribute RT-PCR master mix to a PCR plate or to tubes.
3. Add sample to each reaction.

Component		Volume
RT-PCR master mix	2X RT-PCR Buffer	12.5 µL
	Forward and reverse PCR primers	__ µL
	TaqMan® probes	__ µL
	25X RT-PCR Enzyme Mix	1 µL
	(*Optional)* Detection Enhancer†	(1.67 µL)

Component	Volume
RNA sample (Nuclease-free Water for NTCs)	__ µL
Total volume per reaction	25 µL

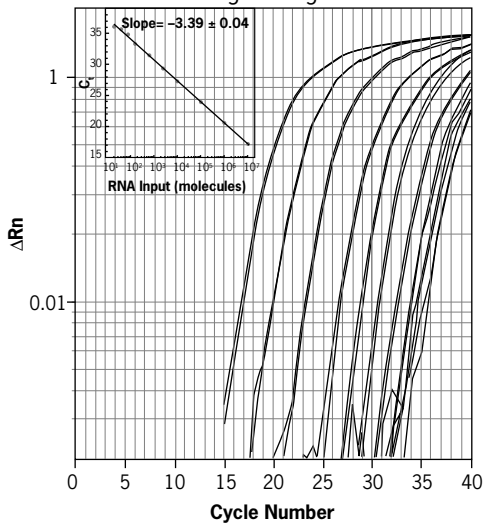
† Try reactions without Detection Enhancer first. Detection Enhancer is recommended only for targets with high GC content and/or persistent secondary structure, and will compromise sensitivity for other targets.

Perform thermal cycling and analyze the data

Run the thermal cycle and analyze RT-PCR data according to the PCR instrument manufacturer's instructions.

The following figure shows amplification of a dilution series of a control RNA sequence using the AgPath-ID™ One-Step RT-PCR Reagents.

Figure 1 Amplification of a Control RNA Using the AgPath-ID™ One-Step RT-PCR Reagents



5 µL of Xeno™ RNA-01 Control dilutions containing 10^7, 10^6, 10^5, 10^4, 2500, 640, 160, 80, 40, and 20 RNA molecules were amplified using the kit on an Applied Biosystems® 7900HT Fast Real-Time PCR System. The amplification plots are shown with an inset showing the linear relationship between C_T and RNA input; the slope is ~3.39 indicating ~100% amplification efficiency.

A

Troubleshooting

Observation	Possible Cause	Solution
No signal from samples expected to be positive	Target sequence has high GC content or persistent secondary structure	Include Detection Enhancer in the RT-PCR master mix. Detection Enhancer may improve amplification with some primer/probe sets.
	RNA sample contains PCR inhibitors	Isolating RNA using the MagMAX™ Kits is typically more effective than glass fiber filter-based RNA isolation methods or TRI Reagent®. Samples containing minimal amounts of inhibitors may yield successful RT-PCRs by adding less sample (and therefore less inhibitor), to the reaction. Alternatively, samples can be diluted, for example 5- and 10-fold, and then used in RT-PCR.
	Problems with RNA isolation	If a carrier RNA was used in viral RNA isolation from cell-free samples, check its concentration to evaluate its recovery.
	For user-designed assays, assay concentration is improperly optimized with RT-PCR Buffer.	Optimize assay concentration.
	Thermal cycler was not properly programmed.	Check programming on thermal cycler.
	RT-PCR master mix setup was incorrect.	Repeat experiment, ensuring master mix setup is correct.
	25X RT-PCR Enzyme Mix was stored improperly and lost activity.	Store reagents as directed.

Observation	Possible Cause	Solution
Signal detected in no template control (NTC)	PCR contamination	Repeat the qRT-PCR reaction with fresh reagents and decontaminated pipettors. Set up and run the qRT-PCR reaction in an area that is isolated from areas used for nucleic acid isolation and PCR product analysis. The Reverse Transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final mastermix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences not contained in the expression vector.

B Supplemental information

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Maintain separate areas and edicate equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (Cat. no. AM9890).

C

Safety

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Documentation and support

Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologiess' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies Corporation at **www.lifetechnologies.com/support**.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

16 March 2015

life technologies

A Thermo Fisher Scientific Brand