AgPath-ID™ One-Step RT-PCR Reagents

USER GUIDE

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

Catalog Numbers AM1005, 4387424, and 4387391

Publication Number 1005M

Revision K

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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. 1005M

Revision	Date	Description
K	14 September 2022	The regulatory statement was updated.
J	27 August 2019	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Removed Detection Enhancer from "Contents and storage" on page 4.
H	19 March 2015	• Clarified that the kit is intended for use with single or duplex assays. • Other format, style, and legal updates.

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

Contents

1 Product information

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

Product description

AgPath-ID™ One-Step RT-PCR Reagents are designed for sensitive, robust amplification of RNA targets using a single-tube TaqMan™-based real-time RT-PCR strategy. Each kit is optimized for use with single or duplex TaqMan™ primer/probe sets.

Each kit includes the following reagents:

- **25X RT-PCR Enzyme Mix:** Contains ArrayScript™ Reverse Transcriptase and AmpliTaq Gold™ DNA Polymerase.
- **2X RT-PCR Buffer:** Includes ROX™ passive reference dye for quantitative fluorescent signal normalizaton.

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

Contents and storage

Catalog numbers that appear as links open the web pages for those products.

Component	Cat. No. AM1005 (100 reactions)	Cat. No. 4387424 (500 reactions)	Cat. No. 4387391 (1,000 reactions)	Storage
2X RT-PCR Buffer	1375 µL	7 mL	14 mL	–10°C to –30°C in a non-frost-free freezer
25X RT-PCR Enzyme Mix	110 µL	550 µL	1100 µL	
Nuclease-free water	1.75 µL	25 mL	25 mL	—

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Real-time PCR system, one of the following:	
QuantStudio™ 5 Real-Time PCR System	Contact your local sales office.
Applied Biosystems™ 7500 Fast Real-Time PCR System	
Applied Biosystems™ 7500 Real-Time PCR System	
Applied Biosystems™ 7900HT Fast Real-Time PCR System	
ABI PRISM™ 7500 Sequence Detection System	
MX3000P QPCR System	Agilent
SmartCycler™ II System	Cepheid
Equipment	
Adjustable pipettors	MLS
Reagents	
***(Optional)* Detection Enhancer**[1]	
Detection Enhancer - 100 rxn	A44810
Detection Enhancer - 500 rxn	A44941
Detection Enhancer - 1000 rxn	A44811
Tubes, plates, and other consumables	
96-well PCR plate or tubes	**thermofisher.com/plastics**
Nuclease-free pipette tips	**thermofisher.com/pipettetips**
Reagent reservoirs or tubes[2]	MLS

[1] Recommended for targets with high GC content and/or persistent secondary structure.
[2] Recommended for preparing the RT-PCR master mix.

Guidelines for input RNA

- Use pure RNA that is free of RT-PCR inhibitors.
- We recommend using a MagMAX™ RNA isolation kit that is appropriate for your sample type. Go to **thermofisher.com**, then search for ***MagMAX***.
- For cell-free sample types, such as serum, we recommend using the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700 or A32702).

Guidelines for PCR primer/TaqMan™ probe mix

- Single and duplex TaqMan™ primer/probe sets must be compatible with your real-time PCR instrument and designed for one-step RT-PCR.
- Optimize the concentrations of primers and probe for your experiment. See Table 1 for recommended starting concentrations.
- The reverse transcriptase that is contained in the kit is produced using an *E. coli* expression vector. The vector contains 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 master mix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequences, we recommend designing primer sequences that are not contained in the expression vector.

Table 1 Recommended PCR Primer/TaqMan™ probe concentrations

Component	Final concentration in the reaction	25X primer/probe mix[1]
Forward PCR primer	400 nM	10 µM
Reverse PCR primer	400 nM	10 µM
TaqMan™ probe	120 nM	3 µM

[1] Use 1 µL of PCR primer/TaqMan™ probe mix per 25-µL RT-PCR reaction.

2 Methods

Set up the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions.

Set up the thermal protocol for your instrument according to one of the following tables.

- Passive reference dye: ROX™ (included in the RT-PCR Buffer)
- Reaction volume: 25 µL

IMPORTANT! If you are using the SmartCycler™ II instrument, set ramp rates to 1.6°C/sec to prevent amplification failure.

Table 2 Thermal protocol: SmartCycler™ II instrument

Step	Stage	Cycles	Temp	Time
Reverse transcription	1	1	45°C[1]	10 min
RT inactivation/initial denaturation	2	1	95°C	15 min
Amplification	3	40	95°C 60°C	15 sec 60 sec

[1] 50°C may be a more effective RT temperature for some PCR primer sets.

Table 3 Thermal protocol: All other compatible instruments

Step	Stage	Cycles	Temp	Time
Reverse transcription	1	1	45°C	10 min
RT inactivation/initial denaturation	2	1	95°C	10 min
Amplification	3	40	95°C 60°C	15 sec 45 sec

Prepare the RT-PCR reactions

- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 5–10% overage, unless otherwise indicated.
- Include duplicate no template controls (NTCs) using nuclease-free water in place of sample.

1. Prepare each RT-PCR master mix on ice according to Table 4.
2. Transfer the appropriate volume of RT-PCR master mix to a PCR plate or tubes.
3. Add samples or controls to the wells or tubes containing RT-PCR master mix (25 µL final volume per reaction).

Table 4 RT-PCR reaction mix volumes

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]	(1.67 µL)
RNA sample (or nuclease-free water for NTCs)		__ µL
Total volume per reaction		**25 µL**

[1] Run the first reaction without Detection Enhancer. Detection Enhancer is recommended only for targets with high GC content and/or persistent secondary structure. Detection Enhancer can compromise sensitivity for other targets. For more information, see Appendix A, “Troubleshooting and FAQs”.

Perform RT-PCR, then analyze the results

Start the run, then analyze the RT-PCR data according to the PCR instrument manufacturer instructions.

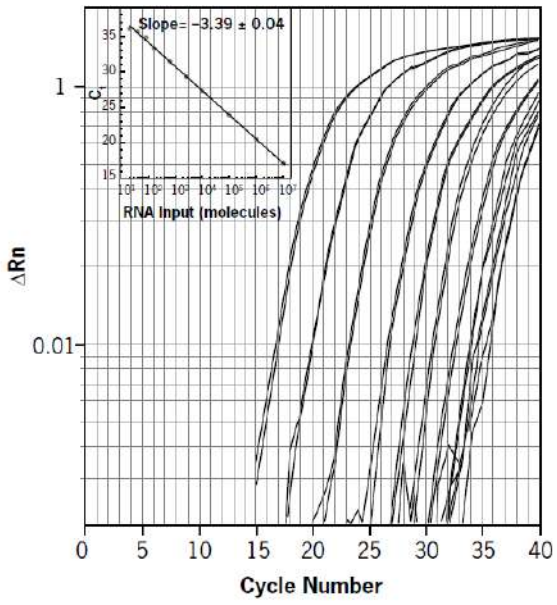

Figure 1 Amplification of a control RNA sequence using AgPath-ID™ One-Step RT-PCR Reagents

For the amplification, 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 that displays the linear relationship between C_t and RNA input; the slope is ~3.39, which indicates ~100% amplification efficiency.

A Troubleshooting and FAQs

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Aminal Health Support Center: **http://thermofisher.com/animalhealthsupport**
- For FAQs for this product: **http://thermofisher.com/am1005faqs**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

B

Supplemental information

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

C Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

D Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - 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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.