USER GUIDE

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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

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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

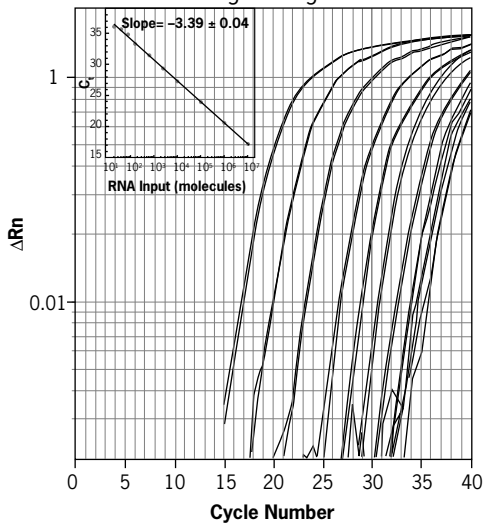
† Try reactions without Detection Enhancer first. Detection Enhancer is recommended only for targets with high GC content and/or persistent secondary structure, and will compromise sensitivity for other targets.

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

TaqMan™ Gene Expression Assays —single-tube assays

USER GUIDE

Publication Number 4333458

Revision R

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. 4333458

Revision	Date	Description
R	17 December 2019	Removed troubleshooting and replaced with link to FAQs; updated multiplex reaction Application Note and url; updated endogenous control reference to Pub. No. COL33019 0619.
Q	15 May 2018	• Updated thermal cycling conditions for TaqMan™ Fast Advanced Master Mix. • Added option of cDNA preamplification. • Add option of C_{rt} algorithm for troubleshooting. • Corrected troubleshooting for inhibitors in the real-time PCR reaction.
P	22 November 2017	• Added new instruments, Master Mixes, and other products applicable for the workflows. • Removed content that is described in other resources; added references as appropriate. • Streamlined and clarified content for ease of use and reading. • Updated for general style, formatting, and branding.
N	November 2010	Baseline for this revision history.

Contents

1 Product information

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

Product description

TaqMan™ Gene Expression Assays are a comprehensive collection of predesigned, preformulated primer and probe sets to perform quantitative gene expression studies on a variety of species. For a current list of available species and assays, use the Assay Search Tool at **thermofisher.com/taqmangeneexpression**.

- **TaqMan™ Gene Expression Assays**
  - A general collection of assays that target protein-coding transcripts from a variety of species and for specific diseases, pathways, or biological processes.
  - TaqMan™ Non-coding RNA Assays that target long non-coding RNA (ncRNA) in human, mouse, and rat species. These assays are designed for ncRNAs that are > 60 nt in length.
- **Endogenous control assays** (see page 18 for more information).

Note: Custom TaqMan™ Gene Expression Assays can also be designed. To design a custom assay, go to **www.thermofisher.com/cadt**. For more information on the design tool, see *Custom TaqMan™ Assays Design and Ordering Guide* (Pub. No. 4367671).

This document provides guidance for preparing cDNA templates (see page 12) and protocols for performing real-time PCR using a variety of compatible instruments and Master Mixes (see page 13).

For detailed information about TaqMan™ Gene Expression Assays, see page 18.

Contents and storage

Item	Storage
TaqMan™ Gene Expression Assay (single-tube format)	–25°C to –15°C[1]

[1] Shipped at ambient temperature. See **thermofisher.com/ambientshippping**.

Go to **thermofisher.com/taqmanfiles**, then enter your order number to download the following files.

- Assay information files (AIFs)
- User Instruction Documents (Protocols, User Guides, and Quick Reference Cards)
- Certificates of Analysis
- Safety Data Sheets

For detailed information about the shipment and assay information files (AIF), see *Understanding Your Shipment* (Pub. No. MAN0017153).

TaqMan™ Gene Expression Assay formulations

To find and order predesigned, preformulated primer and probe sets in a variety of species, go to **thermofisher.com/taqmangeneexpression**, then use the Assay Search Tool.

Table 1 Standard formulations

Product	Dye	Size	Number of 20-µL reactions	Cat. No.	Concentration
TaqMan™ Gene Expression Assays	FAM™	Extra Small	75	4453320[1] or 4448892	20X
		Small	250	4331182[1]	
		Small	360	4351372	
		Medium	750	4351370	
		Large	2900	4351368	60X
	VIC™	Small	360	4448489	20X
		Medium	750	4448490	
		Large	2900	4448491	60X
TaqMan™ Gene Expression Assays, Primer-Limited (PL)	VIC™	Small	360	4448484	20X
		Medium	750	4448485	
		Large	2900	4448486	60X
TaqMan™ Non-coding RNA Assay	FAM™	Small	360	4426961	20X
		Medium	750	4426962	
		Large	2900	4426963	60X

[1] This product is inventoried.

Custom TaqMan™ Gene Expression Assay formulations

Use the Custom TaqMan™ Assay Design Tool (**www.thermofisher.com/cadt**) to enter and submit sequences for new assay design. The tool also supports submission files created using FASTA file format. For details, see the *Custom TaqMan™ Assays Design and Ordering Guide* (Pub. No. 4367671).

For a comparison of Custom and Custom Plus Assay products go to **thermofisher.com/customtaqmangex**.

Table 2 Custom formulations

Product	Dye	Size	Number of 20 µL reactions	Cat. No.	Concentration
Custom Plus TaqMan™ RNA Assay	FAM™	Small	360	4441114	20X
		Medium	750	4441117	
		Large	2900	4441118	60X
	VIC™	Small	360	4448514	20X
		Medium	750	4448515	
		Large	2,900	4448516	60X
Custom PlusTaqMan™ RNA Assay, Primer-Limited (PL)	VIC™	Small	360	4448511	20X
		Medium	750	4448512	
		Large	2900	4448513	60X
Custom TaqMan™ Gene Expression Assays	FAM™	Small	360	4331348	20X
		Medium	750	4332078	
		Large	2900	4332079	60X
	VIC™	Small	360	4448508	20X
		Medium	750	4448509	
		Large	2900	4448510	60X
Custom TaqMan™ Gene Expression Assay, Primer-Limited (PL)	VIC™	Small	360	4448487	20X
		Medium	750	4448488	
		Large	2900	4448492	60X

Assay primer and probe concentrations

Assay type	Concentration					
	Forward primer		Reverse primer		Probe	
	1X	20X	1X	20X	1X	20X
TaqMan™ Gene Expression Assays (FAM™ or VIC™)	900 nM	18 µM	900 nM	18 µM	250 nM	5 µM
TaqMan™ Gene Expression Assays (VIC™, Primer Limited)[1]	150 nM	3 µM	150 nM	3 µM		

[1] Recommended for multiplexing. For more information, see "Guidelines for duplex reactions using TaqMan™ Gene Expression Assays" on page 22.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 3 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	thermofisher.com/rnaisolation

Table 4 Recommended products for preparation of cDNA

Item	Source
cDNA kit or cDNA Master Mix, one of the following:	
SuperScript™ IV VILO™ Master Mix	11756050
SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme	11766050
High-Capacity cDNA Reverse Transcription Kit	4368813

Table 5 PCR Master Mixes

Item	Source
(Recommended) TaqMan™ Fast Advanced Master Mix	4444556
TaqMan™ Gene Expression Master Mix	4369016
TaqMan™ Universal Master Mix II, with UNG	4440038
TaqMan™ Universal Master Mix II, no UNG	4440047
TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG	4352042

Table 6 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	Contact your local sales office
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
StepOne™ or StepOnePlus™ Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
7500/7500 Fast Real-Time PCR System	

Item	Source
Software	
(Optional) Relative Quantification application	Available on the Connect
(Optional) Standard Curve application	
(Optional) ExpressionSuite™ Software	Available at **thermofisher.com/expressionsuite**
Equipment	
Thermal cycler, one of the following (or equivalent): • Veriti™ Thermal Cycler • SimpliAmp™ Thermal Cycler • ProFlex™ PCR System	Contact your local sales office
Centrifuge, with adapter for 96-well or 384-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher Scientific™ 21-379-00
Pipettes	MLS
Tubes, plates, and other consumables	
Tubes, plates, and film	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free Water	AM9930
RNase Inhibitor	N8080119
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
TURBO DNA-*free*™ KitDNase	AM1907
TE, pH 8.0	AM9849
(Optional) TaqMan™ PreAmp Master Mix	4391128
(Optional) TaqMan™ PreAmp Master Mix Kit	4384267

Workflow

Start with cDNA templates prepared from RNA samples (page 12)

▼

Prepare the PCR Reaction Mix (page 14)

▼

Set up and run the real-time PCR instrument (page 15)

▼

Analyze the results (page 16)

2 Guidelines for preparation of cDNA

Guidelines for isolation of high–quality RNA

- See Table 3 on page 9 for recommended RNA isolation kits.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- See Table 4 on page 9 for recommended cDNA synthesis kits.
- Use the same reverse transcription procedure for all samples.
- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

    IMPORTANT! Degradation of the RNA may reduce the yield of cDNA for some gene targets.
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified.
  Use TaqMan™ PreAmp Master Mix (Cat. No. 4391128) or TaqMan™ PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

3 Perform real-time PCR

Procedural guidelines for performing real-time PCR

- Follow best-practices when preparing or performing PCR (see page 22).
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Configure run documents according to the instructions provided in the real-time PCR instrument user documents.
- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- Run technical replicates in triplicate to identify outliers.

Before you begin (60X assays)

Dilute 60X assays to 20X working stocks with TE, pH 8.0, then divide the solutions into smaller aliquots to minimize freeze-thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run. An example dilution is shown in the following table.

1. Gently vortex the tube of 60X assay, then centrifuge briefly to spin down the contents and eliminate air bubbles.

2. In a 1.5-mL microcentrifuge tube, dilute sufficient amounts of 60X assay for the required number of reactions.

Component	Volume
TaqMan™ Gene Expression Assays (60X) or Custom TaqMan™ Gene Expression Assays (60X)	40 µL
TE, pH 8.0 (1X)	80 µL
Total aliquot volume	**120 µL**

3. Store aliquots at –20°C until use.

Prepare the PCR Reaction Mix

Thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Mix the Master Mix thoroughly but gently.

2. Combine the PCR Reaction Mix and assays in an appropriately-sized microcentrifuge tube according to the following table.

Component	Volume for 1 reaction	
	Standard 96–well or 48–well Plates	**384–well Plate or 96–well Fast Plate**
Master Mix (2X)[1,2]	10 µL	5 µL
TaqMan™ Gene Expression Assay (20X) or Custom TaqMan™ Gene Expression Assay (20X)	1 µL	0.5 µL
Nuclease-free water[3]	7 µL	3.5 µL
Total PCR Reaction Mix volume	**18 µL**	**9 µL**

[1] Recommended: TaqMan™ Fast Advanced Master Mix

[2] (*Optional*) If you add AmpErase™ UNG (uracil-N-glycosylase), the final concentration must be 0.01U/ µL. Reduce the volume of water in the PCR reaction mix to compensate for additional volume from the UNG.

[3] Adjust the volume of nuclease-free water for a larger volume of cDNA.

3. Vortex to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.

5. Add cDNA template (1 pg–100 ng in nuclease-free water), or nuclease-free water for NTC, to each well.

   - 1 µL for a 384-well plate or 96-well Fast Plate
   - 2 µL for a 96-well and 48-well Standard Plate

   Note: Be sure to adjust the volume of nuclease-free water in the PCR reaction mix for a larger volume of cDNA.

   IMPORTANT! For optimal results when using TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG, prepare the plate on ice. Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

6. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

Set up and run the real-time PCR instrument

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

Note: The instrument must be configured with the block appropriate for the plate type.

1. Select the cycling mode appropriate for the Master Mix.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

2. Set up the thermal protocol for your instrument.

   See "Thermal protocols" on page 21 for the thermal protocols for other Master Mixes.

   Table 7 TaqMan™ Fast Advanced Master Mix (StepOne™, StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

   [1] Optional, for optimal UNG activity.

   [2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time.

   Table 8 TaqMan™ Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

   [1] Optional, for optimal UNG activity.

   [2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time

3. Set the reaction volume appropriate for the reaction plate.
   - **96-well Standard (0.2-mL) Plate**: 20 µL
   - **96-well Fast (0.1-mL) Plate and 384–well Plate**: 10 µL

4. Load the plate into the real-time PCR instrument.

5. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the absolute or relative quantification ($\Delta\Delta C_t$) methods to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group.

Perform additional analysis using any of the following software:

Software	Resource
Relative Quantification application	**thermofisher.com/cloud**
Standard Curve application	
ExpressionSuite™ Software[1]	**thermofisher.com/expressionsuite**

[1] Can automatically define the baseline. Files from a QuantStudio™ 3 or 5 System are not compatible.

For more information about real-time PCR, see *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239) or go to **thermofisher.com/qpcreducation**.

Troubleshooting and FAQs

Visit our online 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 troubleshooting information and FAQs for this product: **thermofisher.com/taqmangesingletubefaqs**
- To browse the database and search using keywords: **thermofisher.com/faqs**

B

Supplemental information

Endogenous controls

An endogenous control shows gene expression that is relatively constant and moderately abundant across tissues and cell types and treatment protocols. Normalization to endogenous control genes is currently the most accurate method to correct for potential biases that are caused by:

- Sample collection
- Variation in the amount of starting material
- Reverse transcription (RT) efficiency
- Nucleic acid (RNA/DNA) preparation and quality

No single control can act as a universal endogenous control for all experimental conditions, so we recommend verifying the chosen endogenous control or set of controls for the sample tissue, cell, or treatment. See *Using TaqMan™ Endogenous Control Assays to select an endogenous control for experimental studies* (Pub. No. COL33019 0619), available from **thermofisher.com**.

To select and order endogenous control assays, go to **thermofisher.com/taqmancontrols**.

TaqMan™ Gene Expression Assays chemistry overview

TaqMan™ MGB probes

TaqMan™ MGB probes contain:

- A reporter dye (for example, FAM™ dye) at the 5′ end of the probe.
- A non-fluorescent quencher (NFQ) dye at the 3′ end of the probe.
  The NFQ dye does not fluoresce, which allows the real-time PCR system to measure the reporter dye contributions more accurately.
- A minor groove binder (MGB) at the 3′ end of the probe that:
  - Increases the melting temperature (T_m) without increasing the probe length.
  - Allows for the design of shorter probes.

About the 5' nuclease assay

Note: The following figures are general representations of real-time PCR with TaqMan™ MGB probes and TaqMan™ Gene Expression Assays. The sequence regions are not necessarily drawn to scale.

The 5' nuclease assay process takes place during PCR amplification. It occurs in every cycle and does not interfere with the exponential accumulation of cDNA synthesis product.

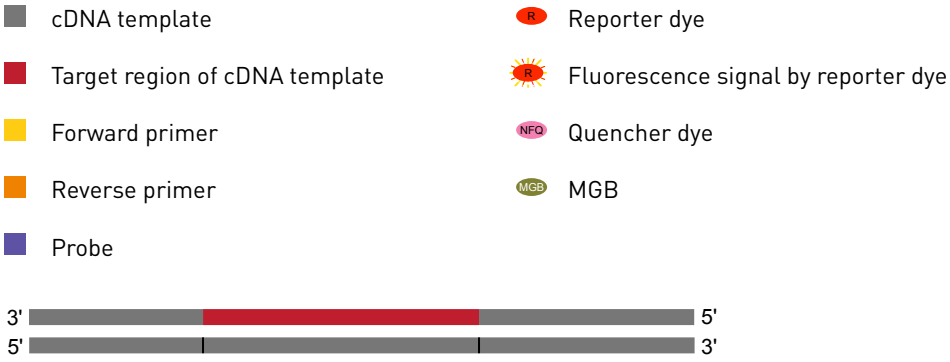


Figure 1 cDNA synthesis product

During the PCR, the forward and reverse primers anneal to complementary sequences along the denatured cDNA template strands (see Figure 2).

The TaqMan™ MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (see Figure 2). When the probe is intact, the proximity of the reporter dye and quencher dye suppresses the reporter fluorescence, primarily by Förster-type energy transfer.

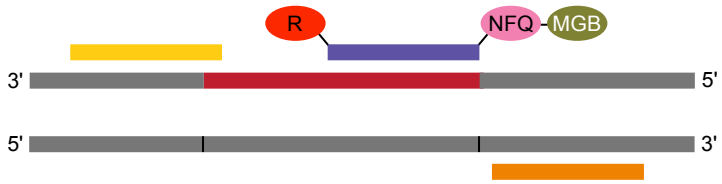


Figure 2 Annealing of probes and primers to cDNA strands

During polymerization, the DNA polymerase only cleaves probes that hybridize to the target sequence. Cleavage separates the reporter dye from the probe. The separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter dye (see Figure 3).

This increase in fluorescence occurs only if the probe is complementary to the target sequence and if the target sequence is amplified during PCR. Because of these conditions, nonspecific amplification is not detected.

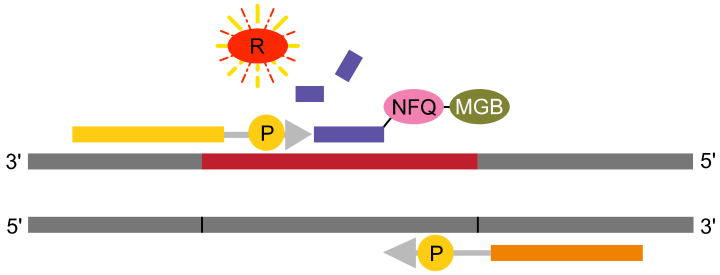


Figure 3 Initial polymerization and cleavage of reporter dye

Polymerization of the strand continues (see Figure 4), but because the 3' end of the probe is blocked, no extension of the probe occurs during PCR.

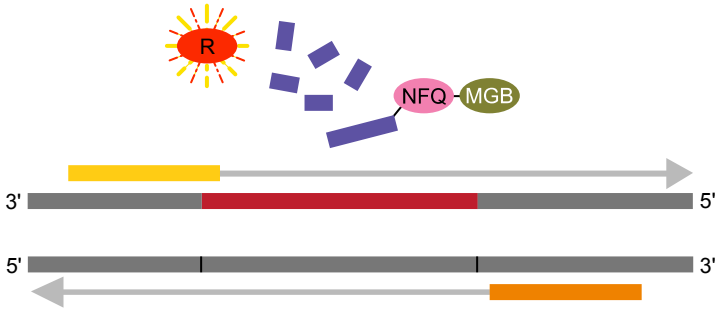


Figure 4 Completion of polymerization

Enzyme activation time

Using TaqMan™ Fast Advanced Master Mix, the enzyme activation step can range from 20 seconds to 2 minutes. A 20–second enzyme activation step is sufficient when the template is cDNA. A longer enzyme activation time will not affect the results.

The enzyme activation time for the default fast thermal cycling conditions on the instruments is 20 seconds. If a longer enzyme activation time is required, change the thermal cycling conditions before starting the run. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.

Algorithms for data analysis

Table 9 Algorithm recommendations for single–tube assays

Algorithm	Recommendation
Threshold (C_t)	Recommended.
Relative threshold (C_{rt})	*(Optional)* Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on Connect (**thermofisher.com/connect**).

Thermal protocols

The thermal protocol settings depend on:

- The real-time PCR instrument
- Whether the Master Mix requires fast or standard cycling mode based on its chemistry
- Whether the Master Mix contains UNG

The thermal protocols in "Set up and run the real-time PCR instrument" on page 15 are optimized for the TaqMan™ Fast Advanced Master Mix.

The following tables provide thermal protocols for other Master Mixes that are compatible with TaqMan™ Gene Expression Assays.

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

Table 10 TaqMan™ Gene Expression Master Mix or TaqMan™ Universal Master Mix II, with UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

[1] For optimal UNG activity.

Table 11 TaqMan™ Universal Master Mix II, no UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

Table 12 TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG (StepOne™, StepOnePlus™, ViiA™ 7, or QuantStudio™ system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 13 TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG (7500 or 7500 Fast system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

Guidelines for duplex reactions using TaqMan™ Gene Expression Assays

Duplex real-time PCR is the simultaneous amplification and measurement of two target sequences in one reaction. TaqMan™ Gene Expression Assays can be used in duplex real-time PCR when using a FAM™ dye-labeled assay in combination with a primer-limited, VIC™ dye-labeled assay. When setting up a duplex reaction:

- Validate that your duplex assay combinations provide similar results to your singleplex reactions.
- Consider the relative expression levels of each target.
- Perform serial dilutions of your sample in both singleplex and duplex reactions, and compare the results for relative expression.
- Select the higher-expressing target as the primer-limited, VIC™ dye-labeled assay.
- Use TaqMan™ Fast Advanced Master Mix, which has been optimized for duplexing reactions.

For more details on how to validate your duplex assay reactions and interpret the results, see *Factors Influencing Multiplex Real-Time PCR Application Note* (Pub. No. 136AP04-01) or go to **thermofisher.com/taqmangeneexpressionduplex**.

Best practices for PCR and RT-PCR experiments

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.

Use UNG to prevent false-positive amplification

Carryover amplicons can result in false-positive amplification during PCR. Use a Master Mix that contains uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during an initial incubation at 50°C. UNG is partially inactivated during the 95°C incubation step for template denaturation and polymerase activation. Because UNG is not completely deactivated during the 95°C incubation, it is important to keep the annealing temperatures greater than 55°C and to refrigerate PCR products at 2°C to 8°C in order to prevent amplicon degradation.

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified.
  UNG-containing Master Mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
- Do not attempt to use UNG-containing Master Mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.

Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no master mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

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, see the "Documentation and Support" section in this document.

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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqMan™ Gene Expression Assays Quick Reference—single-tube assays	4401212
Introduction to Gene Expression Getting Started Guide	4454239
Understanding Your Shipment	MAN0017153
Custom TaqMan™ Assays Design and Ordering Guide	4367671
TaqMan™ Assay Multiplex PCR Optimization User Guide	MAN0010189
TaqMan™ PreAmp Master Mix User Guide	4384557
TaqMan™ PreAmp Master Mix Quick Reference	4384556
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide	4489822
QuantStudio™ 12K Flex Real–Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
StepOne™ or StepOnePlus™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
ViiA™ 7 Real-Time PCR System	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434

Document	Pub. No.
7500/7500 Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments	4387783
Data analysis	
Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems	4348358
Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems™ 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide	4364014
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t	4347824
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
Applied Biosystems™ Relative Quantitation Analysis Module User Guide	MAN0014820
Applied Biosystems™ Standard Curve Analysis Module User Guide	MAN0014819

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**.

applied biosystems

TaqMan Gene Expression Assay solutions

Proven performance for fast, reliable results

ThermoFisher SCIENTIFIC

The leader in gene expression analysis

We are the leader in gene expression analysis, providing world-class sample preparation with Applied Biosystems™ technologies, real-time PCR using Applied Biosystems™ TaqMan™ or Applied Biosystems™ SYBR™ Green chemistry, and industry-leading real-time PCR instruments and data analysis software.

Applied Biosystems™ TaqMan™ assay technology is the gold standard in performance, quality, and content for gene expression analysis. Developed using long-standing bioinformatic expertise in primer and probe design, and stringent testing across applications and integrated platforms, TaqMan Assays provide you with the most reliable and robust real-time PCR solutions.

With over one and a half million predesigned and preoptimized assays across a growing list of model species, a wide range of formats to scale to your needs, and a robust manufacturing quality system, we have a complete suite of solutions that will enable you to get fast, reliable, and accurate gene expression results.

Contents

TaqMan Gene Expression Assays

Proven 5´ nuclease–based real-time PCR chemistry

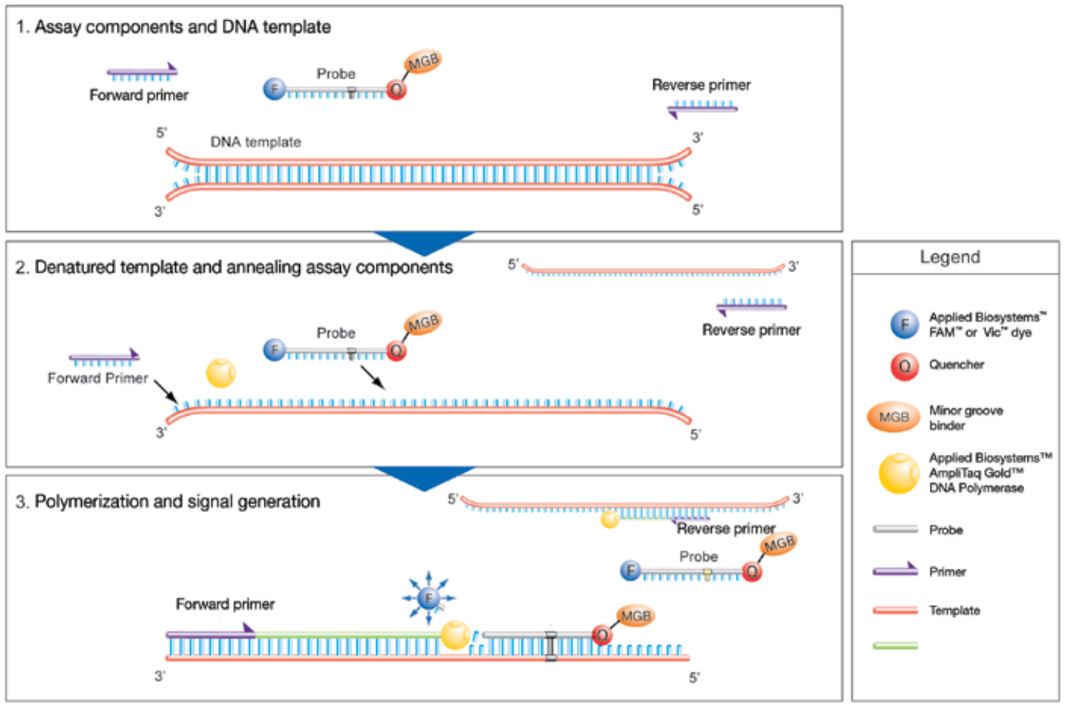
Get results you can trust

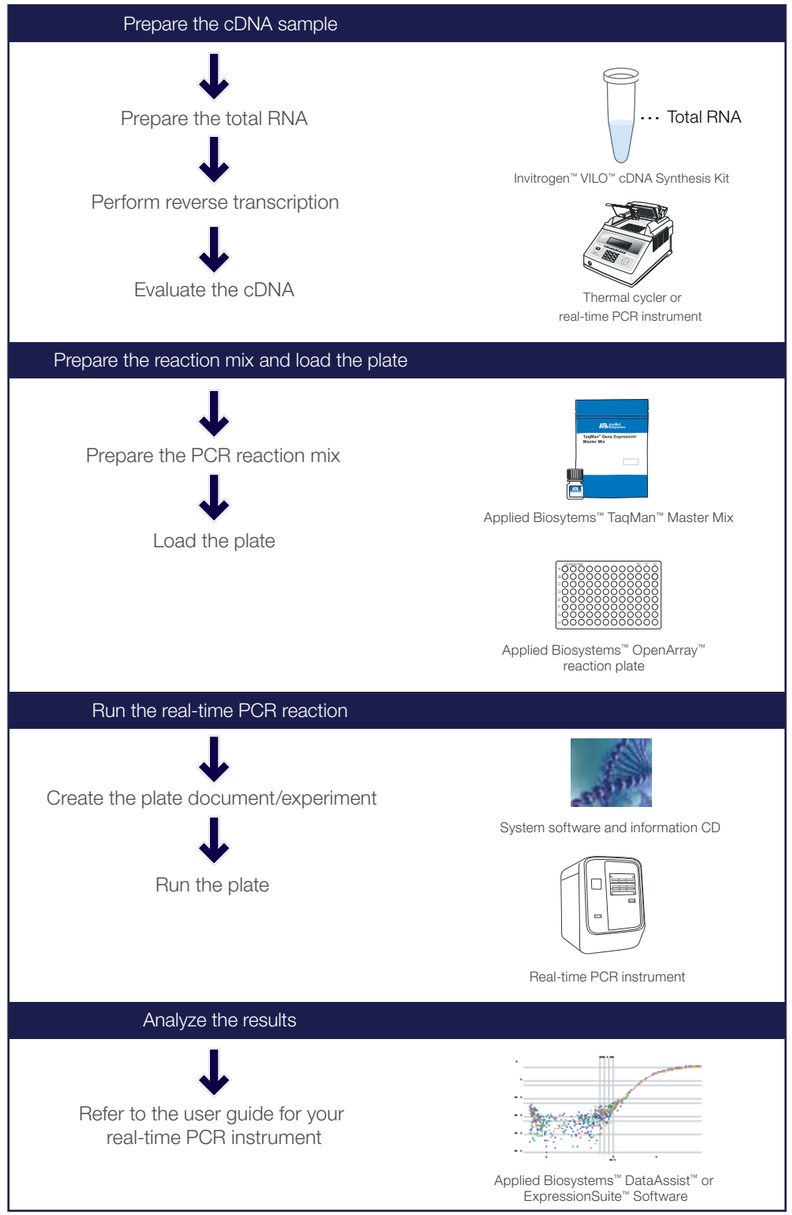
TaqMan Gene Expression Assays are referenced in tens of thousands of publications and are considered the gold standard for gene expression quantification by scientists around the world.

TaqMan Gene Expression Assays are based on 5´ nuclease chemistry, and each assay contains the primer and probe set for your target of interest. Here's how an assay works (Figures 1 - 3):

1. At the start of the real-time PCR reaction, the temperature is raised to denature the double-stranded cDNA. During this step, the signal from the fluorescent dye on the 5´ end of the Applied Biosystems™ TaqMan™ probe is quenched by the MGB–nonfluorescent quencher on the 3´ end of the probe.
2. In the next step, the reaction temperature is lowered to allow the primers and probe to anneal to their specific target sequences.
3. Taq polymerase synthesizes a complementary DNA strand using the unlabeled primers and template. When the polymerase reaches the TaqMan probe, its endogenous 5´ nuclease activity cleaves the probe, separating the dye from the quencher.

With each cycle of PCR, more dye molecules are released, resulting in an increase in fluorescence intensity proportional to the amount of amplicon synthesized.





The largest selection of predesigned assays

Spend time on results, not assay design and optimization

With TaqMan predesigned assays, spend your time generating results, not designing and optimizing assays.

- Detect virtually any gene product—more than 1.5 million predesigned assays, and custom design for everything else
- Assays for nearly every human, mouse, and rat gene in the RefSeq database
- Available for 25 species, and some pathogens
- Assays for multiple locations per transcript and across nearly every exon junction in human
- Strain-neutral assays for mouse and rat

To learn more and order, go to **thermofisher.com/taqmangex**

- Not finding what you're looking for in our predesigned assay collection? The Applied Biosystems™ Custom TaqMan™ Assay Design Tool lets you design and order a TaqMan Assay to detect any gene from any organism. Design and order your assays at **thermofisher.com/cadt** Custom TaqMan Assays are typically delivered in 5–12 business days.
- Also, try Applied Biosystems™ TaqMan™ Endogenous Controls—a collection of TaqMan Assays targeting commonly used control gene products for sample input normalization in real-time PCR.

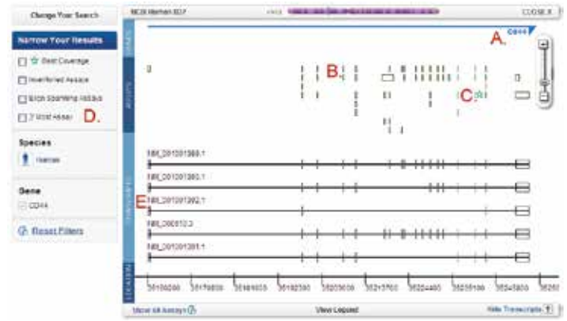
Predesigned TaqMan Gene Expression Assays
(as of November 2015)

Species	Number of assays	Gene coverage (%)*
Human *(H. sapiens)*	205,707	99.8%
Mouse *(M. musculus)*	176,510	99.5%
Chinese hamster *(C. griseus)*	154,743	88.2%
Rat *(R. norvegicus)*	146,589	89.2%
Cow *(B. taurus)*	103,562	99.6%
Rice *(O. sativa)*	99,822	95.6%
Arabidopsis *(A. thaliana)*	97,879	93.8%
Nematode *(C. elegans)*	92,687	95.1%
Rhesus monkey *(M. mulatta)*	69,310	55.8%
Zebrafish *(D. rerio)*	63,712	77.3%
Frog *(X. tropicalis)*	56,764	87.3%
Dog *(C. familiaris)*	55,558	64.3%
Chicken *(G. gallus)*	48,432	85.1%
Fruit fly *(D. melanogaster)*	41,607	94.0%
Sweet corn *(Z. Mays)*	38,493	59.5%
Cynomolgus monkey *(M. fascicularis)*	37,652	80.5%
Pig *(S. scrofa)*	16,247	90.3%
Fission yeast *(S. pombe)*	6,538	94.3%
Rabbit *(O. cuniculus)*	5,927	80.9%
Baker's yeast *(S. cerevisiae)*	5,524	93.4%
Horse *(E. caballus)*	3,891	72.8%
Soybean *(G. max)*	3,456	13.5%
Guinea pig *(C. porcellus)*	2,037	64.3%
Grape *(V. vinifera)*	965	25.3%
Wheat (T. aestivum)	760	43.6%
Summary	**1,534,372**	**81.1%, 25 species**

*Percent coverage refers to genes in the RefSeq database.

There are multiple assays for my gene product. How do I choose the right one?

Genomic alignment maps on our website make it easy to see exactly what gene products are detected and how they align to the genomic locus. The top of the map shows the target gene. Below it, all TaqMan Gene Expression Assays for target gene products are shown relative to the genomic locus map. The known transcripts from the locus are shown below, with their RefSeq accession numbers.



A. Gene symbol

B. Alignment of TaqMan amplicons to the gene. Hover over an assay to see its name and assay number as well as the transcripts it detects. Click on an assay to open an assay details pane for more information and to add the assay to your shopping cart.

C. Assays providing the best coverage are marked with a star symbol.

D. Narrow your results by specifying the type of assay you need.

E. All RefSeq transcripts that map to the gene locus, showing exon usage

The TaqMan Assays qPCR guarantee

We stand behind every predesigned TaqMan Assay. We are committed to helping you achieve your research goals and believe our predesigned TaqMan primer and probe sets establish the benchmark for high-quality and easy-to-use real-time PCR products.

We want you to be happy with your purchase and confident in the genomic tools we provide. Therefore, we guarantee every TaqMan Assay in terms of:

- **Quality**—high-quality manufacturing for reproducible results from lot to lot
- **Performance**—superior sensitivity, specificity, and accuracy
- **Content**—the largest collection of primer and probe sets using the world's best and most extensively validated assay design pipeline
- **Results**—enables you to obtain data you can trust

If you are not satisfied with the performance of a predesigned TaqMan Assay, we'll replace it at no cost or credit your account. For more information, and to see the full terms and conditions of the guarantee, go to **thermofisher.com/taqmanguarantee**

Proven performance

Reliable reagents for confidence in your results

TaqMan MGB probes bind more tightly—shorter, more specific probes

TaqMan probes include an MGB moiety at the 3′ end that increases the T_m of the probe and stabilizes probe–target hybrids. This means that TaqMan probes can be significantly shorter than traditional probes, providing better sequence discrimination and flexibility to accommodate more targets.

Nonfluorescent quencher (NFQ) maximizes sensitivity

TaqMan probes incorporate an NFQ to absorb (quench) signal from the fluorescent FAM or VIC dye label at the other end of the probe. The properties of the NFQ combined with the short length of MGB probes result in lower background signal than with non-MGB/NFQ probes. Lower background noise results in increased sensitivity and precision in your data.

TaqMan probe outperforms non-MGB probe in real-time PCR

	C_t		Standard deviation	
Input	TaqMan Assay	Non-MGB assay	TaqMan Assay	Non-MGB assay
10 ng	9.72	13.35	0.02	0.15
1 ng	13.36	16.82	0.04	0.18
0.1 ng	16.76	20.23	0.07	0.13
10^{-2} ng	20.19	23.72	0.04	0.13
10^{-3} ng	23.64	27.31	0.03	0.10
10^{-4} ng	27.01	30.66	0.04	0.12
10^{-5} ng	30.24	32.82	0.13	0.19

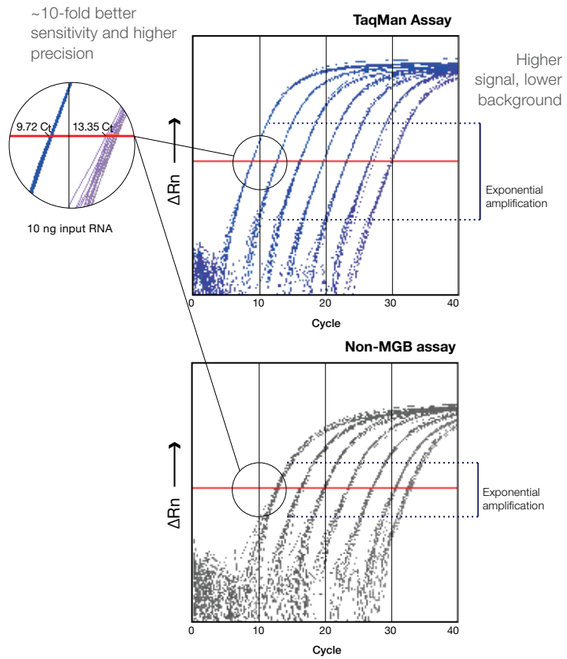


Figure 2. TaqMan probes provide better sensitivity and precision. Comparison of two 5′ nuclease PCR assays for 18S rRNA. Ten-fold dilutions of Universal Human Reference RNA (10–10^{-5} ng) were prepared and analyzed in 11 replicate real-time PCR reactions using either the TaqMan Gene Expression Assay (FAM dye–labeled, with NFQ) or the non-MGB assay (FAM dye–labeled, with BHQ). Real-time PCR was run according to the respective manufacturers' recommended conditions. Across a 6-log range of input template, the TaqMan Assay displayed earlier C_t values and better reproducibility across all data points. In addition, the TaqMan Assay had higher signal and lower background, resulting in better sensitivity and higher precision.

- **Specificity:** Advanced primer/probe sequence selection criteria plus MGB probe enhancement deliver the specificity and reproducibility you need for confidence in your results. Your results are generated from amplification of the intended target, not from nonspecific dye binding or amplification of closely related genes or pseudogenes.
- **Sensitivity:** The NFQ on TaqMan probes minimizes background, and intelligent PCR primer and probe design maximizes amplification efficiency. Get better sensitivity and accuracy—reliably detect targets present at 10 or fewer copies.
- **Reproducibility:** Accurately reproduce results from well to well, day to day, and lab to lab—even across manufacturing lots.
- **Wide dynamic range:** Detect from a handful to millions of target molecules with the same reaction setup. Capture the full spectrum of expression variability in virtually any experimental scenario.
- **High amplification efficiency:** All TaqMan Gene Expression Assays have a PCR efficiency of 100% (±10%). Use the comparative C_t ($\Delta\Delta C_t$) method of quantification confidently.
- **Ease of use:** All assays use a single, universal thermal cycling profile. Run any assay combination on a single plate. Avoid instrument-programming errors.
- **Comprehensive assay information:** Genomic mapping data are provided prior to purchase.

Detect as few as 10 target molecules with high sensitivity and large dynamic range

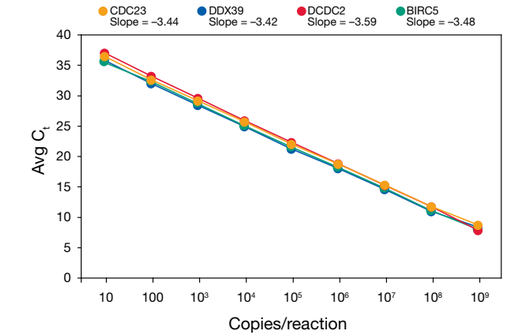


Figure 3. Sensitivity and wide dynamic range. Sequential 10-fold dilutions of synthetic sense RNA corresponding to 4 gene products—CDC23, DDX39, DCDC2, and BIRC5—were added to a background of yeast RNA to evaluate the sensitivity and dynamic range of TaqMan Gene Expression Assays. Samples containing 50 to 5 x 109 target molecules were reverse transcribed, and 20% of each RT reaction was used in quadruplicate PCR reactions using TaqMan Gene Expression Master Mix. Reactions containing as few as 10 copies were detected (C_t ~35).

Reproducible quantification with virtually 100% amplification efficiency

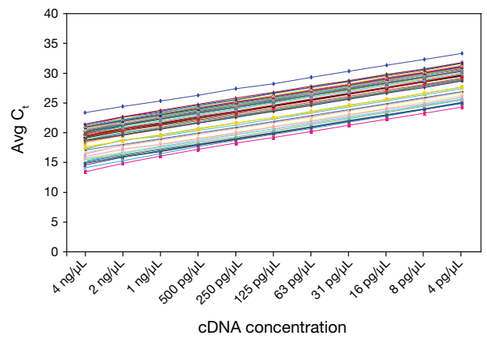


Figure 4. Reliable performance and wide dynamic range. TaqMan Gene Expression Assays were used to analyze expression of 60 targets across a 2-fold dilution series of universal reference cDNA, from 4 ng/µL to 4 pg/µL. The average slope of the lines is 1.02. TaqMan Assays exhibit virtually 100% amplification efficiency at each cycle of PCR: each target molecule is copied, doubling the fluorescence signal.

Specificity for your mRNA target

TaqMan Assay design helps ensure target mRNA specificity: readily distinguish even highly homologous sequences

Specificity is built into the TaqMan Assay design pipeline. As a result, assays detect only their intended targets. Even TaqMan Gene Expression Assays for members of highly homologous gene families typically amplify their targets with C_t values at least 10 cycles earlier than the closest homolog, or with at least 1,000-fold discrimination if equal numbers of the two targets are present.

TaqMan Gene Expression Assays are designed to detect only their intended targets, easily discriminating among highly homologous sequences.

HOX gene family members *HOXA10*, *HOXC10*, and *HOXD10* share ~80% sequence homology

Gene	RefSeq ID	TaqMan Assay ID	Homology
HOXA10	NM_018951.3	Hs00172012_m1	—
HOXC10	NM_017409.3	Hs00213579_m1	81%
HOXD10	NM_002148.3	Hs00157974_m1	79%

Clear gene expression results for HOX gene family members

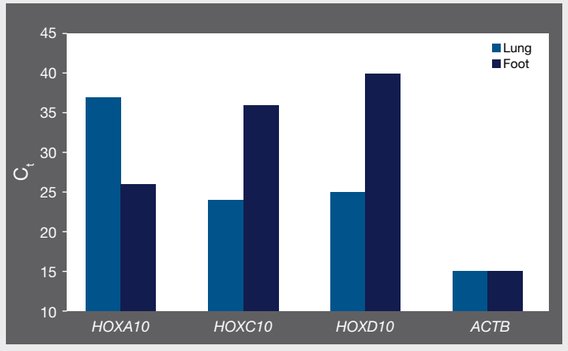


Figure 5. TaqMan Gene Expression Assays detect only their intended targets, even among the highly homologous HOX gene family members. In vertebrates, as in *Drosophila*, location-appropriate expression of members of the HOX gene family is essential for normal embryogenesis. Tissue-specific expression of 3 closely related HOX genes, comparable to published data, was easily detected using TaqMan Gene Expression Assays.

Advanced bioinformatics

TaqMan Gene Expression Assays are designed using our sophisticated design pipeline that has been stringently validated by functionally testing more than 18,000 assays (a statistically significant subset). Since then, our customers have consistently confirmed through their own validation experiments that TaqMan Gene Expression Assays enable reliable, reproducible results.

This process is used to design all TaqMan Gene Expression Assays, including inventoried assays, made-to-order assays, and Applied Biosystems™ Custom Plus assays. We offer ~73,000 inventoried assays and over 1.5 million made-to-order assays, which are manufactured when an order is placed. Applied Biosystems™ Custom Plus TaqMan™ RNA Assays are ideal for newly identified genes and specific splice variants, and offer the same performance as predesigned TaqMan Assays.

TaqMan Assay design and manufacture

Target selection
mRNA sequences (NCBI)

Preprocessing
–Map to genome
–Mask SNPs, repeats, and discrepancies
–Identify exon–exon junction

Assay design
Thermodynamic and chemistry parameters
–Balance T_m for universal thermal cycling
–Avoid secondary structure, optimize GC content
–Optimize amplicon size
–Eliminate primer-dimer formation

***In silico* QC**
–Score assays for target specificity
–Score assays for genome specificity

Assay selection
High-quality TaqMan Gene Expression Assays

Perform stringent assay formulation QC

Confirm oligo identity by mass spectrometry

Online ordering

Flexible formats

A variety of formats for different research needs

Configurations to fit your research goals

Are you analyzing hundreds (or thousands) of samples, and expression from a handful of genes? Or does your research involve a few samples that need to be analyzed for a long list of mRNA targets? No matter what experiment you are performing, there is a TaqMan Gene Expression Assay format and real-time PCR instrument for your research needs.

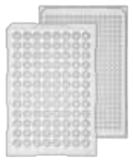
TaqMan Gene Expression Assay formats

Single tubes

- Low entry price
- Flexible
- Run on any real-time PCR instrument

384-well microfluidic cards

- Low cost per reaction
- Optimal for medium to large projects
- Run on Applied Biosystems™ QuantStudio™ 7 & 12K Flex, ViiA™ 7, and 7900HT Real-Time PCR Systems



96- or 384-well plates

- Optimal for small to medium projects
- Balances flexibility with streamlined reaction setup
- Run on any 96- or 384-well real-time PCR instrument

OpenArray plates

- Lowest cost for large projects
- Ultimate throughput
- Run on QuantStudio 12K Flex Real-Time PCR System

TaqMan Gene Expression Assays (single tubes)

Predesigned assays come in four different sizes so that you can order only the number of assays appropriate for your research. In addition, for made-to-order assays in small, medium, and large sizes, you can choose FAM or VIC dye labeling, and non–primer-limited or primer-limited formulation. (Extra small assays are only available with FAM dye labels.)

For more information, go to **thermofisher.com/allgenes**

Size	No. of reactions*	Concentration	Reporter dye	Cat. No.
Extra small (inventoried)†	75	20X	FAM	4453320
Extra small (made-to-order)‡	75	20X	FAM	4448892
Small (inventoried)†	250	20X	FAM	4331182
Small (made-to-order)‡	360	20X	FAM or VIC	4351372, 4448489 (VIC) 4448484 (VIC-PL**)
Medium (made-to-order)‡	750	20X	FAM or VIC	4351370, 4448490 (VIC) 4448485 (VIC-PL**)
Large (made-to-order)‡	2,900	60X	FAM or VIC	4351368, 4448491 (VIC) 4448486 (VIC-PL**)

*Reaction number is based on 20 µL reaction size.
**Primer-limited.
† Inventoried assays are typically delivered in 1–4 business days.
‡ Made-to-order assays are typically delivered in 5–12 business days.

Applied Biosystems™ TaqMan™ Arrays: 96-well plates or 384-well microfluidic cards

- Configure a Custom TaqMan Array containing inventoried predesigned assays, or select from our gene signature assay collections
- TaqMan Gene Expression Assays are loaded into one of two TaqMan Array formats: 96-well plates (Fast or standard) or 384-well microfluidic cards

(To include made-to-order or custom assays on your plate or card, order using our Applied Biosystems™ TaqMan™ Custom Plating Service, or contact your sales representative for other options.)

Custom TaqMan Array 96-well plates

- Choose any inventoried TaqMan Gene Expression Assay
- 6-plate minimum order
- Choose standard (20 µL rxn) or Fast (10 µL rxn) format

Typically delivered in 4–14 business days

To learn more and order, go to **thermofisher.com/arrayplates**

Assays + controls	Assay replicates	Samples per plate	Name	Cat. No. (standard)	Cat. No. (Fast)
95 + 1*	1	1	Format 96	4391524	4413255
92 + 4**	1	1	Format 96 +	4391525	4413256
47 + 1*	2	1–2	Format 48	4391526	4413257
44 + 4**	2	1–2	Format 48 +	4391527	4413258
31 + 1*	3	1–3	Format 32	4391528	4413259
28 + 4**	3	1–3	Format 32 +	4391529	4413260
15 + 1	6	1–6	Format 16	4413264	4413261
12 + 4	6	1–6	Format 16 +	4413265	4413262
7 + 1	12	1–12	Format 8	4413266	4413263

*Available with one manufacturing control assay for 18S ribosomal RNA. These formats are required for plates with assays for rhesus, canine, or a mixture of species.
**Includes the manufacturing control assay for 18S ribosomal RNA, plus assays for 3 additional candidate endogenous control genes: *GAPDH*, *HPRT1*, and *GUSB*, appropriate for human, mouse, or rat sample analysis.

Custom TaqMan Array 384-well microfluidic cards

- Choose any inventoried TaqMan Gene Expression Assays
- 10-card minimum order
- Run on the QuantStudio 7 & 12K Flex, ViiA 7, and 7900HT Fast Real-Time PCR Systems
- No robotics required: cards have 8 sample-loading ports, each connected to 48 wells containing dried-down TaqMan Assays
- 1 µL reactions (2 µL including channel filling and overage)
- Typically delivered in 3–4 weeks

To learn more and order, go to **thermofisher.com/arraycards**

Assays + controls*	Assay replicates	Samples per card	Name	Cat. No.
11 + 1	4	8	Format 12	4342247
15 + 1	3	8	Format 16	4346798
23 + 1	2 (or 4)	8 (or 4)	Format 24	4342249
31 + 1	3	4	Format 32	4346799
47 + 1	1 (or 2)	8 (or 4)	Format 48	4342253
63 + 1	3	2	Format 64	4346800
95 + 1	1 (or 2)	4 (or 2)	Format 96a	4342259
95 + 1	2 (or 4)	2 (or 1)	Format 96b	4342261
191 + 1	2	1	Format 192	4346802
380 + 4	1	1	Format 384	4342265

*These arrays are available with one manufacturing control assay for 18S ribosomal RNA.

Applied Biosystems™ TaqMan™ Array Gene Signature Plates and Cards

- Predesigned, preloaded TaqMan Assays for gene products specific to pathways, biomarkers, or disease target classes to facilitate drug discovery and disease research
- Endogenous control panels are also available to identify the best housekeeping gene products for your research
- Gene signature plates are typically delivered in 5–10 business days, and gene signature cards in 1–4 business days

Here is a sampling of what's available:

– Apoptosis

– Endogenous controls

– Cancer

– Immune system and inflammation

– Cell cycle proliferation and regulation

– Neurology

– Development and stem cells

– Signal transduction

– ECM matrix and adhesion

– Toxicology and drug metabolism

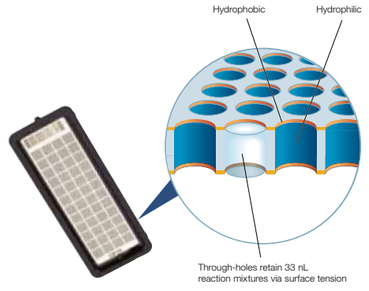
To see the complete collection of 96-well gene signature plates, go to **thermofisher.com/signatureplates**
To see the collection of 384-well gene signature microfluidic cards, go to **thermofisher.com/signaturecards**

OpenArray Real-Time PCR Plates

- TaqMan Assays loaded and dried down into the 3,072 through-holes on OpenArray Real-Time PCR Plates
- Process up to 576 samples to obtain over 43,000 data points, with a single operator in an 8-hour day, without the use of robotics
- For use with the QuantStudio 12K Flex Real-Time System with an Applied Biosystems™ OpenArray™ block configuration and supporting reagent kits only
- OpenArray plates with inventoried assays are typically delivered in 4–5 weeks, and within 5–6 weeks for custom assays

To learn more about OpenArray technology on the QuantStudio 12K Flex system,go to **thermofisher.com/openarray**

Assays + controls	Assay replicates	Samples per plate	Name	Cat. No.
18	3	Up to 48	Format 18	4471124
56	1	Up to 48	Format 56	4471125
112	1	Up to 24	Format 112	4471126
168	1	Up to 16	Format 168	4471127
224	1	Up to 12	Format 224	4471128



TaqMan Custom Plating Service: 96- or 384-well plates

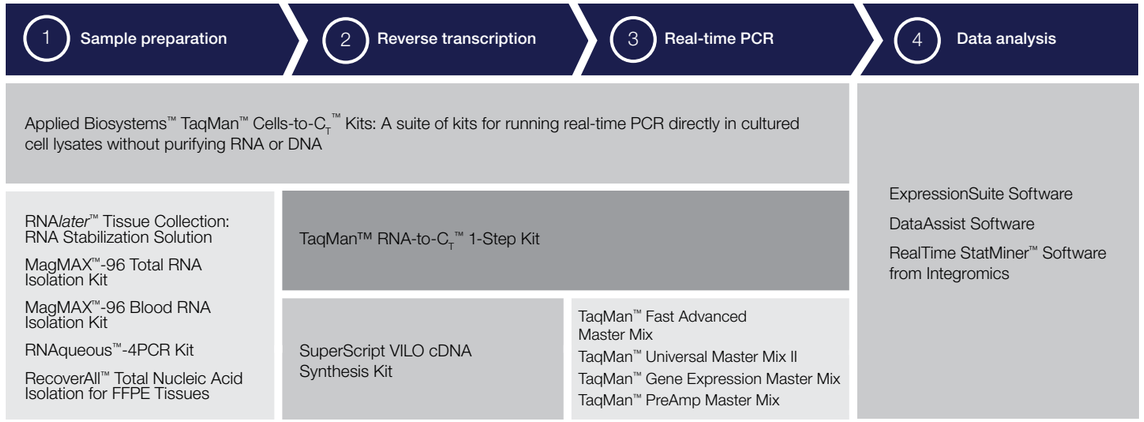
Configure 96- or 384-well plates with any TaqMan Gene Expression Assays, including custom assays designed to your target sequences and made-to-order assays.

- Set up custom configurations of any TaqMan Assays, including inventoried, made-to-order, custom, or Custom Plus gene expression assays or custom TaqMan probes and primers
- Choose 96- or 384-well plate, and Fast or standard format
- Receive in dried-down or liquid formulation
- Typically delivered in 2–5 weeks

Complementary reagents

Everything you need for reliable results

We provide everything you need for real-time PCR analysis, starting with isolating RNA from virtually any sample type, to reverse transcription into cDNA, optional preamplification to stretch small samples for analysis of many gene products, and of course, real-time PCR data analysis.



TaqMan chemistry vs. SYBR Green chemistry for real-time PCR

We offer two types of chemistries to detect PCR products using real-time PCR instruments:

- TaqMan Assay chemistry (also known as “fluorogenic 5´ nuclease chemistry”)
- SYBR Green I dye chemistry

	TaqMan Assay–based detection	SYBR Green–based detection
Overview	Uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles	Uses SYBR Green I dye, or similar: dye binds to double-stranded DNA, to detect PCR product as it accumulates during PCR cycles
Specificity	High	Low
Sensitivity—low copies	High	Variable*
Reproducibility	High	Variable*
Multiplexing	Yes	No
Predesigned assays	Yes	No
User design and optimization	No	Yes
Cost	High	Low*
Gene expression quantitation	High	Low
DNA quantitation	Yes	Yes (pathogen detection)
ChIP	Yes	Yes
SNP genotyping	Yes	No
MicroRNA	Yes	No
Copy number	Yes	No
Somatic mutation detection	Yes	No
Pathway analysis	Yes	No

*Depends on template quality and primer design/optimization.

Support at every step of your workflow

Consistent reliability from manufacturing to follow-up

Quality manufacturing and stringent quality control

TaqMan Assays are manufactured in-house under rigorous quality processes at our ISO 13485–certified manufacturing facilities, and are never outsourced.

Comprehensive worldwide support

Whether you need help finding a TaqMan Assay for your target, deciding which format best suits your needs, placing your order through our online ordering system, or setting up your reactions, our global sales and technical support teams are here to help.

Technical support

If you have questions about how to use TaqMan Assays or how to analyze results, call or email our technical support specialists. These scientists are skilled in experimental planning and design, are expert troubleshooters, and are familiar with a wide variety of applications that use TaqMan Assays.

Rapid delivery

We continually strive to minimize delivery time on TaqMan Assay products. To that end, we have implemented streamlined order processing systems that interface with our new manufacturing facilities to help reduce delivery times.

Everything you need to meet the MIQE guidelines for peer-reviewed publications

The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, published by Bustin et al. in *Clinical Chemistry* (April 2009), are meant to ensure that real-time PCR experiments are meaningful, accurate, and reproducible. We support this initiative and commend the MIQE scientists for their leadership.

We provide the following for easier adherence to these guidelines:

- **TaqMan Assay annotation**—Information requested under the real-time PCR target, oligonucleotide, and protocol sections of the guidelines is provided in your assay shipment and on our website. All biologically relevant information is available, including assay location, transcripts detected, and amplicon size. Protocols with recommended reagents and reaction conditions are also available on our website.
- **Publications**—There are >9,900 peer-reviewed publications that cite TaqMan Assays, so including the TaqMan Assay ID in lieu of sequences is sufficient and widely accepted.
- **Instrument software**—Applied Biosystems™ instrument software reports C_t values for quantification. The C_t can be used to generate standard curves, determine slope, and derive R2 values. To help adhere to the MIQE guidelines, the term quantification cycle (C_q) may be used directly in place of C_t.
- **Data analysis**—We offer data analysis software, including ExpressionSuite and DataAssist Software; simple-to-use tools for calculating relative gene expression using statistical analysis and visualization; and RealTime StatMiner Software (Integromics) for additional statistical analysis workflows.

applied
biosystems

Find out more at **thermofisher/allgenes**

ThermoFisher
SCIENTIFIC

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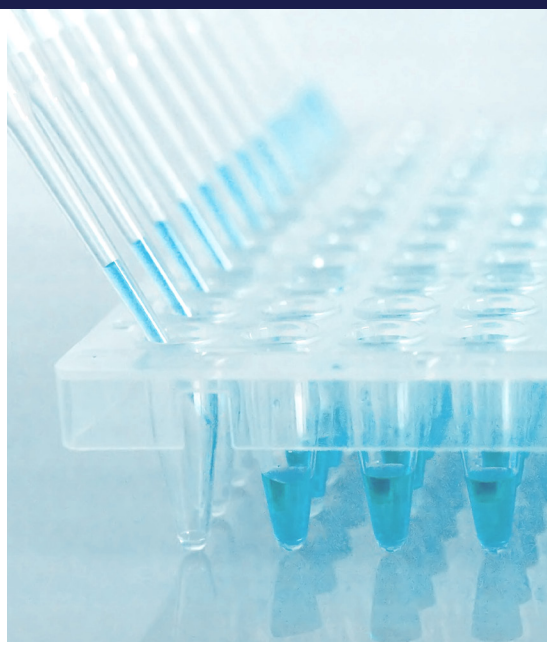

Master mixes built for the specific needs of veterinary labs

Introduction

The value of your PCR testing results is only as good as the reagents you rely on. That's why our enzymes have been optimized to help you identify the animal pathogen targets most important to you. We have rigorously developed reagents that are robust and consistent, with the ability to perform in the presence of PCR inhibitors found in even the most challenging animal samples. Whether your lab's needs are simple or complex, or whether you are new to PCR testing or have been designing veterinary assays for years, our easy-to-use master mixes can help you feel confident in your results (Figure 1).

Our offering includes:

- One-step RT-PCR master mix
- Multiplex one-step RT-PCR master mix
- Fast-cycling multiplex one-step RT-PCR master mix
- Master mixes with internal positive control (IPC)

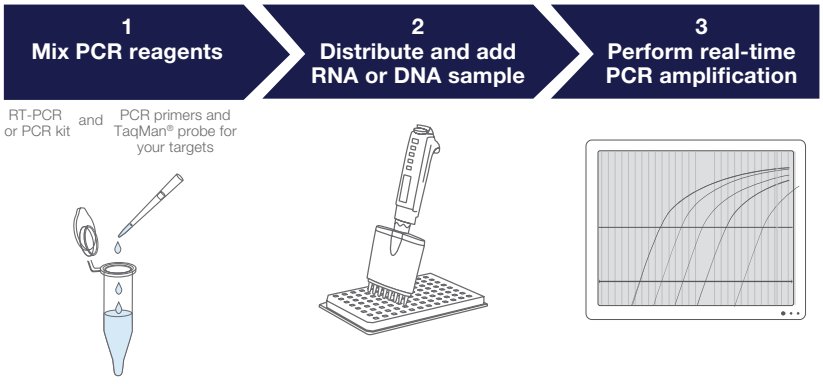

Figure 1. Workflow for our master mix products.

ThermoFisher SCIENTIFIC

One-step RT-PCR master mix

Applied Biosystems™ AgPath-ID™ One-Step RT-PCR Kit—economical, high-quality, ready-to-use master mix for amplification of RNA targets.

- Consistent, reliable amplification helps provide results you can trust
- Simple single-tube, one-step reaction minimizes handling and helps reduce the risk of cross-contamination
- Detection enhancer provided as an optional reagent for amplification of difficult templates

Formulation

The AgPath-ID One-Step RT-PCR Kit is designed for sensitive, robust amplification of RNA targets in the presence of PCR inhibitors typically found in animal samples. The kit includes:

- 25X RT-PCR enzyme mix containing:
  - Invitrogen™ ArrayScript™ Reverse Transcriptase (RT), a mutant M-MLV RT that produces high cDNA yields
  - Ultrapure hot-start DNA polymerase providing superior specificity and sensitivity
- Optimized 2X RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes Invitrogen™ ROX™ dye as an internal reference for normalization and precise data analysis
- Detection enhancer as an optional reagent for amplification of templates with high GC content or persistent secondary structure

Sensitive, reliable performance

To illustrate the consistent performance of the AgPath-ID One-Step RT-PCR Kit, serial dilutions of virus A control RNA containing 5 to 5 x 10^6 copies were amplified (Figure 2). The amplification plot shows a consistent set of curves expected from highly efficient PCR, and the graph shows the reliability and efficiency of the reaction across a wide range of input template amounts.

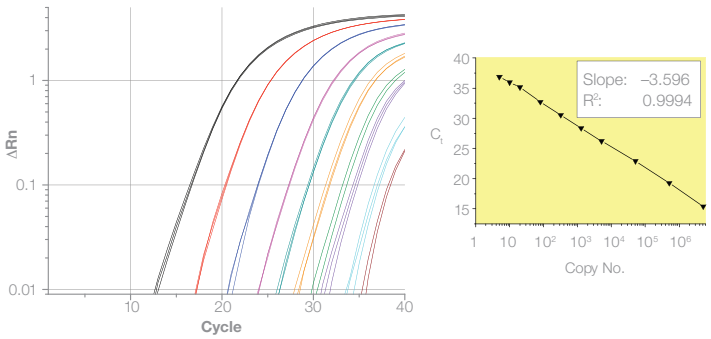

Figure 2. qRT-PCR targeting serially diluted virus A control RNA transcript (5 to 5 x 10^6 copies) demonstrates highly efficient and consistent performance of the AgPath-ID One-Step RT-PCR Kit.

Figure 3 shows amplification of a serial dilution of a different control RNA, virus B. Amounts of RNA were kept low (20 to 40,000 copies) in order to compare the analytical sensitivity of target amplification of the AgPath-ID kit and a competitor's RT-PCR kit. The AgPath-ID One-Step RT-PCR Kit provided earlier C_t values and better analytical sensitivity than the competitor's kit across the dilution range.

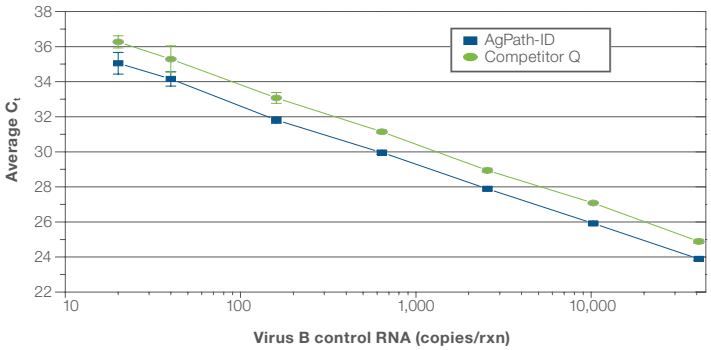

Figure 3. AgPath-ID One-Step RT-PCR Kit is more sensitive than a leading competitor's kit. Serially diluted virus B control RNA (20 to 40,000 copies) was amplified using the AgPath-ID One-Step RT-PCR Kit and a leading competitor's kit.

Multiplex one-step RT-PCR master mix

Applied Biosystems™ Path-ID™ Multiplex One-Step RT-PCR Kit—highly sensitive and convenient master mix optimized for veterinary labs targeting RNA pathogens.

- Simultaneous multiplex amplification of up to 4 different targets helps save time and money
- Optimized to amplify low–copy number (20 copies) targets to deliver results even with challenging samples
- Capable of amplification of over 7 logs of input to provide robust performance when you need it

Formulation

The Path-ID Multiplex One-Step RT-PCR Kit is designed for the sensitive, robust amplification and multiplex quantitation of animal pathogen RNA in a simple format. The kit includes:

- Multiplex enzyme mix containing:
  - An M-MLV RT capable of producing high cDNA yields
  - Ultrapure hot-start DNA polymerase providing superior specificity and sensitivity
- Multiplex RT-PCR Buffer with optimized reagents for efficient, robust results from both the reverse transcription reaction and the PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis

Multiplex with confidence

In the study depicted in Figure 4, the Path-ID Multiplex One-Step RT-PCR Kit provides higher target analytical sensitivity in comparison to a competitor's product.

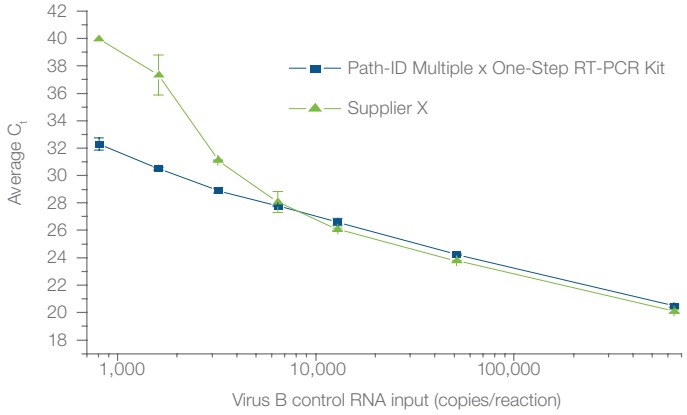

Figure 4. The Path-ID Multiplex One-Step RT-PCR Kit amplifies the lower amounts of target with better sensitivity (lower C_t values) than the competitor kit. A quadraplex RT-PCR experiment was performed using the Path-ID Multiplex One-Step RT-PCR Kit and a competitor kit. Only data for the virus B target are shown.

Figure 5 shows that the Path-ID Multiplex One-Step RT-PCR Kit comparably amplifies targets in singleplex and duplex RT-PCR reactions, suggesting that there is no loss of sensitivity as a result of multiplexing.

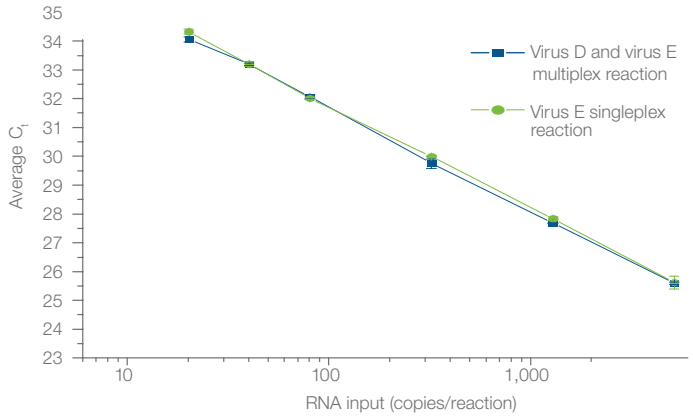

Figure 5. The Path-ID Multiplex One-Step RT-PCR Kit shows no difference in sensitivity between singleplex and multiplex reactions. Virus E RNA was reverse-transcribed and PCR-amplified in a singleplex reaction, and virus D RNA and virus E RNA were reverse-transcribed and coamplified in a duplex reaction, using the Path-ID Multiplex One-Step RT-PCR Kit for both reactions.

Figure 6 shows the amplification of 4 targets by multiplex RT-PCR using the Path-ID Multiplex One-Step RT-PCR Kit. The quantities of 3 of the targets in the experiment were held constant, but the fourth target was serially diluted to show the dynamic range of multiplex target amplification with the kit. The results show that the Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies 4 animal pathogen RNA targets in a single reaction.

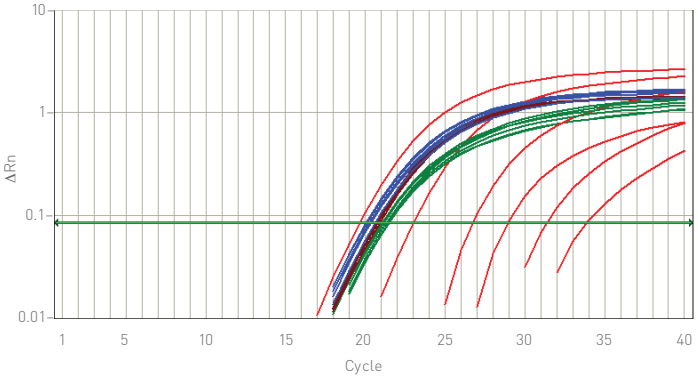

Figure 6. The Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies multiple pathogen targets in a single reaction. Applied Biosystems™ Xeno™ RNA Control and control RNAs for virus A, virus B, and virus C were amplified in a single multiplex reaction using the Path-ID Multiplex One-Step RT-PCR Kit. A sample set with fixed amounts of 3 of the targets and a serial dilution series of the virus B control RNA (red curve) were included.

Fast multiplex one-step RT-PCR master mix

Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix—fast, reliable, highly sensitive real-time RT-PCR even in the presence of common reaction inhibitors.

- One-tube, one-step 4x master mix to amplify both RNA and DNA with high sensitivity
- Capable of working with singleplex or multiplex targets and with exogenous or endogenous internal controls
- Increased qRT-PCR speed on fast and on standard instruments

Formulation

With the TaqMan Fast Virus 1-Step Master Mix you can perform reverse transcription and PCR all in one reaction well. It includes:

- AmpliTaq™ Fast DNA Polymerase UP, for rapid hot-start PCR
- A rapid thermostable M-MLV RT for high sensitivity on viral nucleic acid targets
- Additives to greatly improve success using samples that contain RT-PCR inhibitors such as blood, anticoagulants, dirt, and feces
- A buffer solution that does not freeze at the –20°C storage temperature

Fast cycling and flexiblility

The TaqMan Fast Virus 1-Step Master Mix helps speed your time-to-results and maximizes the use of your real-time PCR instruments. The 4X formulation allows for more target nucleic acid sample to be added to the smaller reaction volumes (required for fast protocols). This enables you to maintain sensitivity with low-titer samples, while improving speed and throughput. Figure 7 shows the experiment times for four Applied Biosystems RT-PCR kits. The fast cycling capabilities of TaqMan Fast Virus 1-Step Master Mix allows for twice as many runs as can be completed with a standard cycling reagent in the same amount of time. Additionally, compared to other one-step kits, the single-tube format of the TaqMan Fast Virus 1-Step Master Mix saves hands-on time.

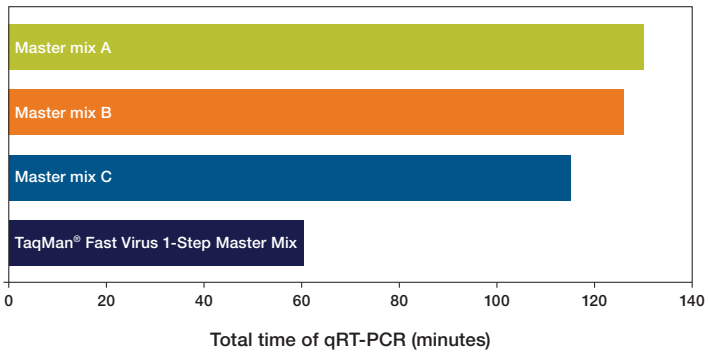

Figure 7. TaqMan Fast Virus 1-Step Master Mix can perform twice as many runs as standard cycling reagents. Experiment times for four Applied Biosystems RT-PCR kits were compared using the same instrument (Applied Biosystems™ 7500 Real-Time PCR System). All master mixes tested were run according to their recommended cycling times and conditions.

qPCR master mix

Applied Biosystems™ Path-ID™ qPCR Master Mix—highly sensitive master mix used to detect animal pathogen DNA, optimized to perform in the presence of challenging qPCR inhibitors.

- Capable of amplifying over 7 logs of input and down to 25 copies of target for dependable, robust performance
- Inhibitor tolerance to help deliver accurate results even with challenging samples
- Stable performance at a wide temperature range allows for convenient reaction setup and reagent storage

Formulation

Path-ID qPCR Master Mix is designed for the sensitive, robust amplification of animal pathogen DNA in a convenient format. It includes:

- Ultrapure hot-start DNA polymerase enabling room temperature reaction setup and minimizes nonspecific PCR products
- Optimized buffer and dNTPs for enhanced sensitivity and functionality in the presence of PCR inhibitors
- ROX dye as an internal reference for normalization and precise data analysis

Convenience and performance

The Path-ID qPCR Master Mix provides dependable target amplification over a linear dynamic range of 6 orders of magnitude, down to 25 copies of target (Figure 8). Path-ID qPCR Master Mix enables amplification of even the most dilute samples.

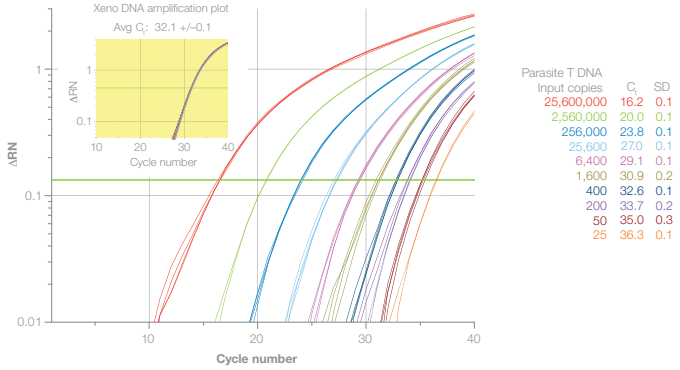

Figure 8. An amplification plot for parasite T DNA in 4 replicate reactions using Path-ID qPCR Master Mix demonstrates that even the most dilute samples are easily amplified. All reactions showed consistent amplification of Xeno DNA Control, an internal positive control (inset).

Path-ID qPCR Master Mix provides reliable amplification of numerous animal pathogen DNA targets in the presence of PCR inhibitors frequently associated with agricultural samples. Figure 9 shows the ability of Path-ID qPCR Master Mix to tolerate high levels of both hematin (20 µM) and humic acid (15 ng/µL) compared to a competitor's master mix.

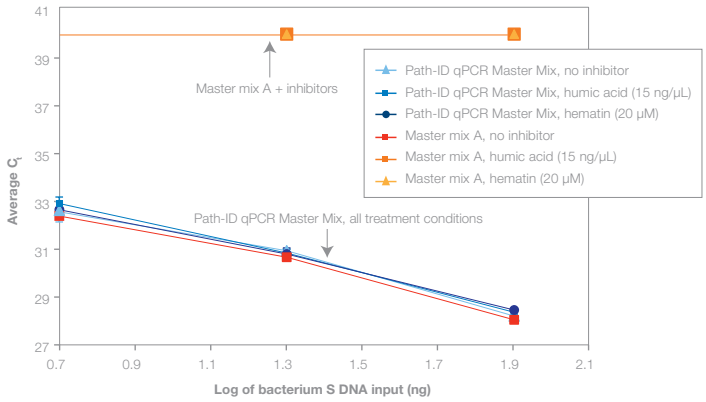

Figure 9. Path-ID qPCR Master Mix shows better tolerance to inhibitors than the competitor's master mix. C_t values are shown for amplification of a dilution series of bacterium S target DNA in the presence of PCR inhibitors, hematin (20 µM) and humic acid (15 ng/µL). The limit of detection for C_t is set at 40.

Path-ID qPCR Master Mix retains high performance even after exposure to harsh conditions. In Figure 10, Path-ID qPCR Master Mix was subjected to multiple freeze/thaw cycles as well as room temperature treatment. In all cases, Path-ID qPCR Master Mix demonstrates equivalent amplification, exhibiting its stability during harsh storage events and even room temperature reaction setup.

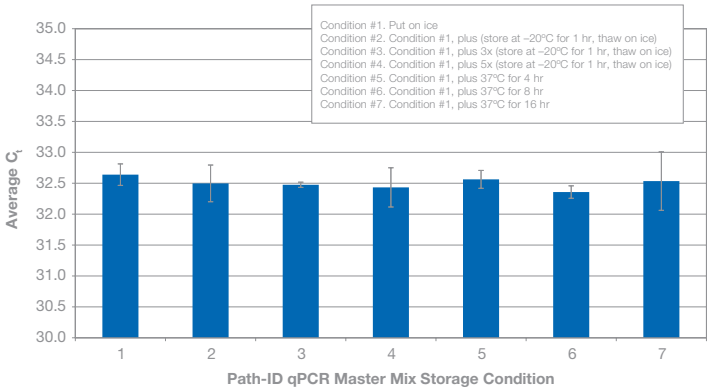

Figure 10. C_t values are given for amplification of bacterium M DNA using Path-ID qPCR Master Mix with various handling conditions. PCR was performed on bacterium M DNA using Path-ID qPCR Master Mix that had been subjected to various freeze/thaw cycles and stored at 37°C for different lengths of time.

Master mixes with internal positive control

Applied Biosystems™ VetMAX™-Plus master mixes provide the highly sensitive and robust performance you need with the added confidence and convenience of a Xeno™ internal positive control (IPC). The use of an IPC in pathogen detection workflows allows you to distinguish true target negatives from PCR inhibition.

- Xeno IPC monitors the reaction for inhibition and effectiveness of nucleic acid purification, enabling greater confidence in results
- Formulations are optimized for use in detecting challenging animal RNA or DNA pathogens
- A suite of master mix options (RT-PCR, multiplex, qPCR) are available to fit your unique application

Formulations

Components of each Applied Biosystems™ VetMAX™-Plus kit are provided below.

Applied Biosystems™ VetMAX™-Plus One-Step RT-PCR Kit

- 25X RT-PCR enzyme mix containing:
  - ArrayScript Reverse Transcriptase, a mutant M-MLV RT that produces high cDNA yields
  - Ultrapure hot-start DNA polymerase providing superior specificity and sensitivity
- 2X RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

Applied Biosystems™ VetMAX™-Plus Multiplex One-Step RT-PCR Kit

- 10X multiplex enzyme mix containing:
  - An M-MLV RT capable of producing high cDNA yields
  - Ultrapure hot-start DNA polymerase providing superior specificity and sensitivity
- 2X multiplex RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

Applied Biosystems™ VetMAX™-Plus qPCR Master Mix

- 2X qPCR master mix containing:
  - Ultrapure hot-start DNA polymerase enables room temperature reaction setup and minimizes nonspecific PCR products
  - Optimized buffer and dNTPs for enhanced sensitivity and functionality in the presence of PCR inhibitors
  - ROX dye as an internal reference for normalization and precise data analysis
- Xeno DNA Control

Qualified results

Using Xeno IPC effectively monitors for PCR inhibition, which means that you can easily qualify your testing results. Figure 11 shows how Xeno IPC identifies the presence of a PCR inhibitor (hematin) at multiple concentrations. The data show that Xeno IPC follows the target's trend of increasing Ct values due to inhibition and therefore can be used as an indicator of inhibition in the reaction. Since the expected range of Xeno IPC Ct values in a normal reaction (without inhibition) is known, you can determine the effect that inhibition has on the reaction, thereby lowering the risk of false negative results.

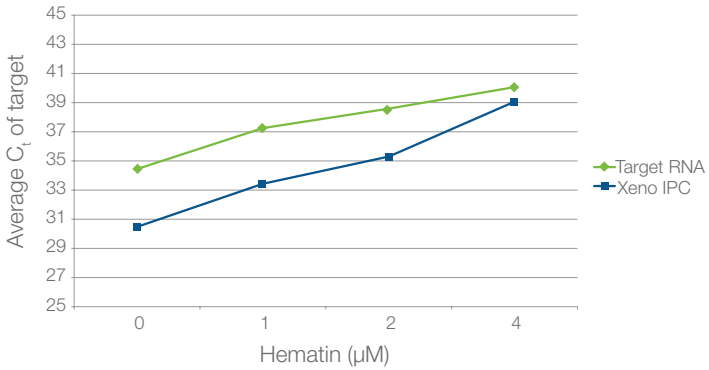

Figure 11. Graph depicting the effect of increasing inhibition on RNA target and subsequent effect on Xeno IPC. 100 copies per reaction of RNA target and 1,000 copies per reaction of Xeno IPC were exposed to increasing levels of hematin (0–4 µM).

For greater quality and consistency of animal RNA and DNA pathogen detection, use VetMAX-Plus master mixes with VetMAX™ reagents and controls.

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Ordering information

Product	Quantity	Cat. No.
Path-ID qPCR Master Mix	100 reactions	4388643
Path-ID qPCR Master Mix	500 reactions	4388644
AgPath-ID One-Step RT-PCR Kit	100 reactions	AM1005
AgPath-ID One-Step RT-PCR Kit	500 reactions	4387424
AgPath-ID One-Step RT-PCR Kit	1,000 reactions	4387391
Path-ID Multiplex One-Step RT-PCR Kit	100 reactions	4442135
Path-ID Multiplex One-Step RT-PCR Kit	500 reactions	4442136
Path-ID Multiplex One-Step RT-PCR Kit	1,000 reactions	4442137
VetMAX-Plus One-Step RT-PCR Kit	100 reactions	4415328
VetMAX-Plus Multiplex One-Step RT-PCR Kit	100 reactions	4415330
VetMAX-Plus qPCR Master Mix	100 reactions	4415327
TaqMan Fast Virus 1-Step Master Mix	200 reactions	4444432
TaqMan Fast Virus 1-Step Master Mix	1,000 reactions	4444434
TaqMan Fast Virus 1-Step Master Mix	2,000 reactions	4444436

Find out more at **thermofisher.com/animalhealth**

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