

# Path-ID™ qPCR Master Mix Protocol

TaqMan® probe-based real-time PCR detection of DNA targets

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## Safety information

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**Note:** For general safety information, see this Preface and [Appendix A, “Safety” on page 5](#). When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

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### Safety alert words


Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:

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**IMPORTANT!** – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.


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 **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.


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 **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

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 **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

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### MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see [“MSDSs” on page 5](#).

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**IMPORTANT!** For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

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## How to use this guide

### User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

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**Note:** – Provides information that may be of interest or help but is not critical to the use of the product.

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**IMPORTANT!** – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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## How to obtain support

For the latest services and support information for all locations, go to:

[www.appliedbiosystems.com](http://www.appliedbiosystems.com)

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

# Path-ID™ qPCR Master Mix Protocol

## Product information

- Purpose of the product** Path-ID™ qPCR Master Mix is a convenient enzyme, buffer, and dNTP mix for TaqMan® probe-based real-time PCR detection of DNA targets, optimized for targets of importance in animal health. With the exception of a PCR primer/TaqMan probe mix for your target, the Path-ID qPCR Master Mix includes everything you need for real-time PCR amplification, including:
- AmpliTaq Gold® ultrapure thermostable DNA polymerase designed for automatic hot start, to minimize nonspecific PCR products
  - Optimized buffer and dNTP concentration, for reliable PCR
  - Passive internal reference, based on proprietary ROX™ dye, for precise data analysis

### Kit contents

Component	100 reactions (PN 4388643)	500 reactions (PN 4388644)
2X qPCR Master Mix	1.375 mL	7 mL
Nuclease-free Water	1.750 mL	25 mL

### Storage

Store all components in a –20 °C non-frost-free freezer.

## Materials and equipment required

**DNA sample(s)** Use pure nucleic acid that is free of PCR inhibitors. To isolate nucleic acid for this procedure, Applied Biosystems recommends using the MagMAX™ Nucleic Acid Isolation Kits. Search for “MagMAX” at [www.appliedbiosystems.com](http://www.appliedbiosystems.com).

**PCR primer/TaqMan® probe mixture** Use any PCR primer/TaqMan probe mixture that is compatible with your real-time PCR instrument. The concentration of primers and probes may require optimization, but those shown in the table below typically work well.

Component	Final concentration in the qPCR
Forward PCR Primer	400 nM
Reverse PCR Primer	400 nM
TaqMan® Probe	120 nM

**Real-time PCR instruments and accessories** The Path-ID qPCR Master Mix is compatible with the following Applied Biosystems PCR systems.

- 7500 System
- 7500 Fast System
- 7900HT System (96-well and 384-well Block)
- 7900HT Fast System (96-well and 384-well Block)
- StepOne™ System
- StepOnePlus™ System

Use reaction plates and accessories appropriate for your real-time PCR system. See the [Plastic Consumables Compatibility Chart](#), available at [www.appliedbiosystems.com](http://www.appliedbiosystems.com) (select Products ▶ Real-Time PCR ▶ Reaction Plates & Adhesive Films).



## Procedure

For the following hazards, see the complete safety alert descriptions in “[Chemical alerts](#)” on page 6:



**WARNING! CHEMICAL HAZARD. 2× qPCR Master Mix.**

1. Program the real-time PCR instrument.

Use the cycling conditions shown.

- ROX™ passive reference dye is included in the 2× qPCR Master Mix.
- The reaction volume is 25 µL.

	Stage	Reps	Temp	Time
Enzyme activation / template denaturation	1	1	95 °C	10 min
Amplification	2	40	95 °C	15 sec
			60 °C	60 sec

2. Assemble the qPCR assays.

- a. Prepare the qPCR cocktail containing 2× qPCR Master Mix, PCR primer/TaqMan probe mixture, and Nuclease-free Water (see table below). Assemble the qPCR cocktail on ice or at room temperature.

- Prepare 5–10% extra qPCR cocktail.
- Negative controls: include duplicate no-template controls using Nuclease-free Water in place of sample.

- b. Distribute the qPCR cocktail to a PCR plate or tubes.

- c. Add the sample to each reaction and mix well.

Component	Amount
2× qPCR Master Mix	12.5 µL
PCR primer/TaqMan® probe mixture	--- µL
DNA sample‡ (Nuclease-free Water for controls)	--- µL
Nuclease-free Water	to 25 µL

‡ Applied Biosystems recommends using <1 µg of input DNA.

3. Perform thermal cycling and analyze the qPCR data.

Follow the PCR instrument manufacturer’s instructions.

## Troubleshooting

Observation	Possible cause	Solution
No signal from samples expected to be positive	DNA sample contains PCR inhibitors	<ul style="list-style-type: none"> <li>• Use less starting sample as input for your DNA isolation procedure.</li> <li>• Increase the number or stringency of washes during DNA isolation.</li> <li>• Use less DNA sample in the PCR. Follow the guidelines below.</li> </ul>
Low signal from samples expected to be positive	DNA sample contains low level of PCR inhibitors	<p>Samples containing minimal amounts of inhibitors may yield successful qPCR reactions if less DNA sample (and therefore less inhibitor) is added to the reaction. For example:</p> <ul style="list-style-type: none"> <li>• Reduce the sample volume to 1–2 µL, and add Nuclease-free Water to bring the reaction to the proper volume.</li> <li>-Or-</li> <li>• Dilute the DNA sample 1:10 using the solution used to elute the nucleic acid at the end of the nucleic acid isolation procedure, and use the diluted DNA in the qPCR reaction.</li> <li>-Or-</li> <li>• Dilute the DNA sample 1:10 using 10 mM Tris-HCl pH 8, 0.1 mM EDTA) and use the diluted DNA in the qPCR reaction.</li> </ul>
Signal detected in no-template control (NTC)	PCR contamination	<ul style="list-style-type: none"> <li>• Repeat the qPCR reaction with fresh reagents and decontaminated pipettors.</li> <li>• Set up and run the qPCR reaction in an area that is isolated from areas used for nucleic acid isolation and PCR product analysis</li> </ul>

## Quality control

### Obtain certificate of analysis

To obtain a Certificate of Analysis for this product, go to [www.appliedbiosystems.com](http://www.appliedbiosystems.com), select **Support** ▶ **Certificates of Analysis**, and search by Part Number/Catalog # and Batch Number/Lot #.

## General chemical safety

### Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” below.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

## MSDSs

### About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

### Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At [www.appliedbiosystems.com](http://www.appliedbiosystems.com), select **Support** ▶ **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At [www.ambion.com](http://www.ambion.com), go to the web catalog page for the product of interest. Select **MSDS**, then right-click to print or download.



- E-mail (MSDS\_Inquiry\_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

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**Note:** For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

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## Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see [“Safety alert words” on page v](#).

### Specific chemical alerts



**WARNING! CHEMICAL HAZARD.** 2× qPCR Master Mix may be harmful by inhalation, in contact with skin and if swallowed. Causes irritation to skin, eyes and respiratory tract. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Wear appropriate protective eyewear, clothing, and gloves.

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