TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix and TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™)

USER GUIDE

Catalog Numbers A58666, A58667, A58668, A58669, A58670, and A58671

Publication Number MAN0028387

Revision B.0

For Laboratory Use.

ThermoFisher SCIENTIFIC

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Revision history: MAN0028387 B.0 (English)

Revision	Date	Description
B.0	2 January 2024	• Product information was updated ("Product description" on page 6). • Additional dye information was added. • Instructions for ordering assays was updated ("Order TaqMan™ Gene Expression Assays" on page 22). • Storage conditions were updated. • Materials were added and updated ("Required materials not supplied" on page 9). The following sections were added: • Appendix A, "Troubleshooting and FAQs" • Appendix B, "Master mix components" • Appendix C, "Assay design guidelines" • Appendix D, "Experimental design guidelines" • Appendix E, "Recommended practices for PCR and RT-PCR experiments" • Appendix G, "Definition of symbols"
A.0	5 July 2023	New document for the TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix and the TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™).

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

Contents

1 Product information

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

Product description

Applied Biosystems™ TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is a benchtop-stable, single-tube master mix optimized for rapid, sensitive, and reproducible detection of viral and bacterial pathogens even in the presence of PCR inhibitors. The 4X concentrated formulation can multiplex six targets in a single well and is well suited for developing high-throughput, molecular diagnostic or bioprocess quality control assays. TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is available in two formulations:

- TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (containing ROX™ passive reference dye)
- TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™)

The TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is designed to perform one-step reverse transcription real-time PCR applications with any gene-specific primer and probe sets for the following types of runs:

- Presence / absence
- Relative quantification
- Standard curve

The master mix is designed for the following applications:

- Multiplex reactions up to six targets (see Table 1 on page 7)
- Use of RNA or DNA as a starting material
- Both the reverse transcription and PCR steps in the same reaction, without affecting DNA targets
- Assembled reaction can be stored at room temperature for up to 8 hours
- Workflows requiring benchtop stable reagents
- Tolerance to common real-time PCR inhibitors

The master mix is supplied at a 4X concentration. It contains the following components:

- AmpliTaq™ DNA Polymerase with Hot-start
- Thermostable MMLV enzyme
- dNTPs including dATP, dGTP, dCTP, dTTP, dUTP
- Recombinant ribonuclease inhibitor
- ROX™ passive reference dye (not included in TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™))
- Buffer components optimized for maximum sensitivity and tolerance to several common RT-PCR inhibitors

Recommended master mix formulation

Table 1 Recommended master mix formulation for singleplex and multiplex reactions

PCR option[1]	Compatible reporter dyes	Recommended master mix formulation
Singleplex (1 probe)	FAM™ dye, VIC™ dye, ABY™ dye	Either of the following master mixes: • TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix • TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™) The master mix with ROX™ dye allows for a passive reference measurement.
Multiplex (2-5 probes, without JUN™ dye)	FAM™ dye, VIC™ dye, ABY™ dye, Cyanine5 dye, Cyanine5.5 dye	
Multiplex (2-5 probes, with JUN™ dye)	JUN™ dye, FAM™ dye, VIC™ dye, ABY™ dye, Cyanine5 dye, Cyanine5.5 dye	TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™) The master mix with ROX™ dye cannot be used if JUN™ dye is a reporter dye. These dyes use the same optical channel.
Multiplex (6 probes)	JUN™ dye, FAM™ dye, VIC™ dye, ABY™ dye, Cyanine5 dye, Cyanine5.5 dye	TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™) The master mix with ROX™ dye cannot be used because the channel is required for a reporter dye.

[1] For detailed information about multiplex reactions, see the *TaqMan™ Assay Multiplex PCR Optimization Application Guide* (Pub. No. MAN0010189).

Contents and storage

Table 2 TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix

Cat. No.[1]	Contents	Number of 20–µL reactions	Storage[2]
A58666	1 x 0.5 mL	100	–25°C to –15°C
A58667	5 x 1 mL	1,000	
A58668	1 x 10 mL	2,000	

[1] Catalog numbers that appear as links open the web pages for those products.
[2] See packaging for expiration date.

Table 3 TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™)

Cat. No.[1]	Contents	Number of 20–µL reactions	Storage[2]
A58669	1 x 0.5 mL	100	–25°C to –15°C
A58670	5 x 1 mL	1,000	
A58671	1 x 10 mL	2,000	

[1] Catalog numbers that appear as links open the web pages for those products.
[2] See packaging for expiration date.

The master mix is shipped on dry ice. The master mix freezes under the shipping conditions but it will be unfrozen at the storage conditions (between -25°C to -15°C).

Note: Under colder storage conditions, the master mix might appear unfrozen or partially frozen. It can be crystallized or semi-solid. Set the master mix on the benchtop or on ice to allow it to return to a liquid state. Mix gently to homogenize.

The master mix can be stored at 2–8°C for up to one month. It can be stored at room temperature (15–24°C) for up to 1 week.

The master mix is supplied with a minumum of 12 months usable shelf-life when stored at –25°C to –15°C.

Required materials not supplied

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

Table 4 Recommended products for isolation of RNA or DNA

Item	Source
Isolation kits	
MagMAX™ CORE Nucleic Acid Purification Kit	A32702
MagMAX™ Prime Viral/Pathogen NA Isolation Kit	A58145
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A48310
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Other nucleic acid isolation or purification kits	**thermofisher.com**

Table 5 Required materials not supplied

Item	Source
One of the following Applied Biosystems™ instruments:	
• QuantStudio™ 5 Dx Real-Time PCR System • QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 7 Pro Dx Real-Time PCR System • QuantStudio™ 7 Pro Real-Time PCR System Or any other compatible real-time PCR system	**https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-instruments/quantstudio-systems.html**
Software	
Diomni™ Enterprise Software Solution Or any other compatible real-time PCR software	**http://thermofisher.com/diomni**
Equipment	
Centrifuge with adapter for 96- or 384-well plates	MLS
Laboratory mixer (vortex mixer or equivalent)	MLS
Microcentrifuge	MLS
Pipettors	MLS

Item	Source
Plastics and other consumables	
Plates and seals for your instrument	**thermofisher.com/plastics**
Disposable gloves	MLS
Pipette tips with filters	MLS
Polypropylene tubes	MLS
Reagents and kits	
Nuclease-Free Water (not DEPC-Treated)	4387936
TE, pH 8.0, RNase-free	AM9849
TaqMan™ Gene Expression Assays	**thermofisher.com/taqmangeneexpression**
RT-PCR Grade Water	AM9935
Cleaning agents or equivalent	
DNA*Zap*™ PCR DNA Degradation Solutions	AM9890
RNase*Zap*™ RNase Decontamination Solution	AM9780

Workflow

TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix

Perform assay screening and multiplex assay optimization

See *TaqMan™ Assay Multiplex PCR Optimization Application Guide* (Pub. No. MAN0010189).

See "Guidelines for multiplexing" on page 26.

Prepare the samples

See Chapter 2, "Guidelines for preparation of nucleic acid".

Prepare the RT–PCR reaction mix (page 14)

Set up and run the real–time PCR instrument (page 16)

Analyze the results (page 17)

2 Guidelines for preparation of nucleic acid

Starting template

The master mix can be used with both RNA and DNA targets.

The reverse transcription step does not affect thermal cycling performance with DNA targets.

Guidelines for preparation of high–quality nucleic acid samples

- For recommended kits to isolate RNA or DNA, see Table 4 on page 9.
- Store isolated nucleic acid at –80°C to –20°C.
- The recommended viral RNA isolation kits include carrier RNA to maximize RNA recovery.

Sample storage conditions

Store the prepared RNA samples at –80°C in the elution buffer provided by the RNA isolation kit or in RT-PCR Grade Water. Store DNA samples at –20°C in the elution buffer provided by the DNA isolation kit or in TE buffer.

If you dilute your samples, it is recommended to use TE buffer or RT-PCR Grade Water as the diluent.

3 Methods

General guidelines

Prevent contamination

Use stringent laboratory practices to avoid false positives that arise through the amplification of contaminants.

For guidelines on recommended laboratory procedures, see Appendix E, “Recommended practices for PCR and RT-PCR experiments”.

Procedural guidelines

- 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.
- Thaw the assays on ice or the benchtop, then mix thoroughly.
- It is recommended to keep RNA samples on ice during the reaction setup.
- It is recommended to use TE buffer or Nuclease-Free Water (not DEPC-Treated) to dilute samples or to prepare the standard dilution series.
- The master mix can be kept on ice or on the benchtop during the reaction setup.
- Mix the master mix thoroughly but gently.
- For applications including automation, the master mix is stable on the benchtop (15–24°C) for most workflows. The viscosity of the master mix at 4X is: 4.69 mPa*s at 22°C and a density of 1.1 mg/mL.
- The master mix is optimized for use with multiplex assays of up to six targets without a passive reference dye. It is optimized for use with multiple assays of up to five targets with the ROX™ passive reference dye.

  See Table 1 on page 7.

Assays and thermal cycling conditions

The master mix is optimized for use with primers and hydrolysis probes.

Optimize and validate each assay independently to ensure appropriate performance. Validate multiplexed assays and re-optimize your thermal cycling conditions as needed.

Note: The term *assay* refers to the primer and probe set.

See "Guidelines for multiplexing" on page 26.

For guidelines on designing multiplex reactions, see *TaqMan™ Assay Multiplex PCR Optimization Application Guide* (Pub. No. MAN0010189).

Guidelines for mixing

The recommended procedure for the preparation of the RT–real-time PCR reaction mix is to mix all of the components including the sample in a tube, then transfer the components to a plate. This requires a separate mixture for each RNA or DNA sample.

The procedure can be modified to mix the master mix, the assay, and the water in a tube. These components can be added to a plate, then the RNA or DNA sample can be added to each well of the plate.

To mix the contents of the reaction plate, seal the reaction plate with an optical adhesive cover and invert the plate three to five times. Ensure that the contents of the wells are moving back and forth between the seal and the bottom of the wells for proper mixing. Centrifuge the plate briefly to pool the liquid to the bottom of the wells.

The master mix is a 4X formulation which can increase viscosity. Ensure that all of the components are thoroughly mixed in all the wells before proceeding to the RT-PCR. Inverting the plate provides a more uniform mixing across the reaction plate than vortexing provides, but a combination of inverting and vortexing is also an option.

Prepare the RT–PCR reaction mix

Thaw RNA samples on ice. Thaw the assays on ice or on the benchtop. Remove the master mix from the freezer and place on ice or on the benchtop.

1. Vortex assays briefly to mix, then centrifuge to collect.
2. Mix thawed samples by gentle inversion or flicking 3–5 times, then briefly centrifuge to collect the contents at the bottom of the tube.
3. Ensure that the reagents are thawed.

   Repeat step 1 and step 2, if required.
4. Calculate the total volume required for each reaction component according to the following table.

   Note: It is recommended to include extra volume to compensate for the volume loss that occurs during pipetting. For example, include 10% overage.

Reaction volumes are scalable. Volumes larger that 30 µL may require reduced ramping speed and increased hold times, depending on the consumable and the instrument used.

Component	Volume (one 20–µL reaction)	Volume (10 × 20–µL reactions)[1]	Notes
TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (4X)	5 µL	55 µL	—
User-defined assays (primers and probe, 20X)[2,3]	1 µL	11 µL	It is recommended to use primer concentrations of 150–900 nM and a probe concentration of 100–250 nM.[4]
RNA or DNA sample	≤14 µL	≤154 µL	Use as much sample as required, up to 70% of the total volume. [5]
RT-PCR Grade Water	Variable	Variable	Fill to the total reaction volume.
Total volume	**20 µL**	**220 µL**	Total reaction volumes other than 20 µL can be used. Ensure that the volumes for each component are scaled appropriately.

[1] Includes 10% overage.

[2] Potential assays include the TaqMan™ Assay Mix, FAM™ dye; TaqMan™ Assay Mix, VIC™ dye; TaqMan™ Assay Mix, ABY™ dye; and TaqMan™ Assay Mix, JUN™ dye, Cyanine5 dye, Cyanine5.5 dye, or other applicable dyes.

[3] Assays can be at a concentration other than 20X. Scale the volume appropriately.

[4] Determine optimal primer and probe concentrations (see “Guidelines for multiplexing” on page 26).

[5] Increasing the sample volumes in the reaction potentially can increase inhibitors.

Note: The components can be mixed together without the RNA or DNA sample, then added to the plate. The RNA or DNA sample can be added directly to the wells of the plate.

For information about mixing, see “Guidelines for mixing” on page 14.

5. Add the combined components directly to each well of an optical reaction plate.

6. Seal the reaction plate with an optical adhesive cover and invert the plate 3–5 times, making sure that the contents of the wells are moving back and forth between the seal and the bottom of the wells to ensure proper mixing.

IMPORTANT! The TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is a 4X formulation. This concentration increases the viscosity. Ensure that all of the components are thoroughly mixed in all the wells before proceeding. Inverting the plate provides a more uniform mixing across the reaction plate than vortexing provides, but a combination of inverting and vortexing is also an option.

7. Centrifuge at 150 × *g* for 1 minute to collect the contents at the bottom of the wells and eliminate air bubbles.

Although, it is recommended to proceed directly to the RT-real-time PCR, the TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is stable in an assembled reaction (all components combined) at room temperature (15–24°C) for up to 8 hours. This enables use with liquid handlers and other benchtop instruments.

Note: Some assays are stable at room temperature for up to 24 hours. Reaction stability at room temperature depends on one or more factors, including sample stability.

Set up and run the real–time PCR instrument

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

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

1. In the real-time PCR system software, set up an experiment using the following parameters:
   - Recommended sample volume: 20 µL
   - Auto Increment Settings: Accept the default value
   - Data Collection: Accept the default value
   - Ramp Rate Settings: Accept the default value

   Note: The volume can be variable.

2. Set up the thermal protocol.

Step	Temperature	Time	Cycles
Reverse transcription[1]	53°C	10 minutes	1
Polymerase activation[2]	95°C	2 minutes	1
Denature	95°C	3 seconds	40
Anneal / extend[3]	60°C	30 seconds	

[1] RT enzyme will function best in the range of 48–55°C.
[2] Required for RT inactivation and initial denaturation, and to activate the DNA polymerase.
[3] Ensure that the annealing temperature is consistent with the melting temperature (T_m) of your primer designs.

3. Select the passive reference dye.

Option	Description
ROX	TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix
None	TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix (No ROX™)

4. Load the reaction plate into the real-time PCR system.

5. Start the run.

4 Analyze the results

For more information about data analysis, see the appropriate documentation for your assay and instrument. Use the standard curve method or the relative quantification ($\Delta\Delta C_t$) method to analyze results.

The real-time PCR system software can be used to set the baseline and threshold values for the amplification plot. They can be set automatically or manually.

The baseline is the initial cycle in which there is a change in the fluorescence signal.

The intersection of the threshold and the amplification plot defines the C_t value. In real-time PCR assays, the threshold is set above the background signal and within the exponential growth phase of the amplification curve.

The general guidelines for analysis include:

- View the amplification plot. Then, if needed:
  - Adjust the baseline and threshold values.
  - Review replicates and outliers.
- In the well table or results table, view the C_t (C_q) values for each well and for each replicate group.

For standard curve experiments, view the following items:

- Slope
- Amplification efficiency
- R^2 value
- Y-intercept
- C_t values
- Outliers

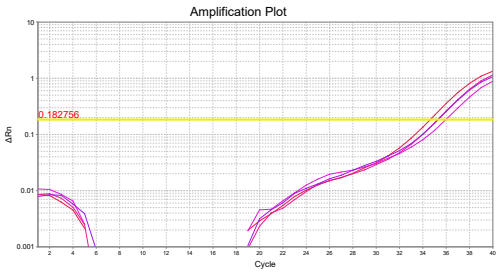
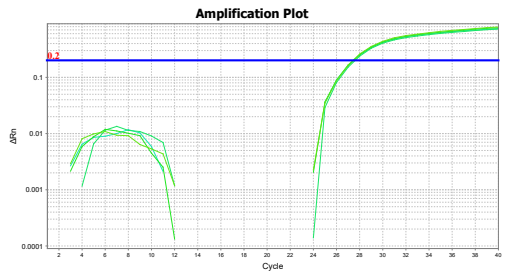
For more information about real-time PCR, see *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239) or go to **www.thermofisher.com/qpcreducation**.

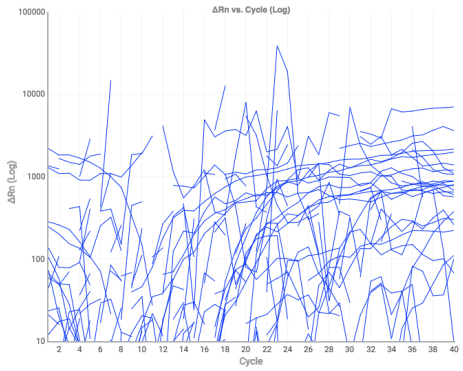
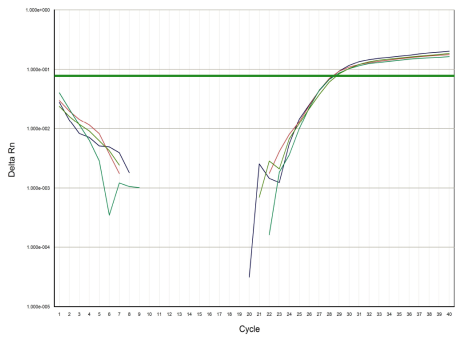
A Troubleshooting and FAQs

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.

To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

This section provides troubleshooting information for the TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix. For general real-time PCR troubleshooting information, see the appropriate guide for your instrument.

Observation	Possible cause	Recommended action
The amplification plot displays S-shaped curves. 	One of the following: • The RT-PCR mix was not thoroughly combined. • Variable florescent baseline.	When preparing the RT-PCR mix, be sure to invert the reaction plate firmly 3–5 times to mix before centrifuging (see “Prepare the RT–PCR reaction mix” on page 14). Add a 10 minute 85°C hold after the reverse transcription step in the thermocycling protocol.
The amplification plot is truncated. 	The baseline was set too high.	When analyzing the data, manually reset the baseline, or use the automatic baseline function (see Chapter 4, “Analyze the results”).

Observation	Possible cause	Recommended action
Amplification plot shows a high level of noise when the No-ROX mix is used. 	ROX is set as the passive reference in the run file.	Change the passive reference to "None" and reanalyze the data.
The automatic threshold is not set in the exponential phase of the amplification curve. 	When automatic baseline is used, the software raises the threshold bar to avoid the elevated baseline.	When analyzing the data, change analysis setting to allow manual adjustment of the threshold, then manually adjust the threshold bar (see Chapter 4, “Analyze the results”). Evaluate the multi-component plot to ensure that the C_t signal represents a true amplification and not part of the background signal noise.

B

Master mix components

AmpliTaq™ DNA Polymerase with Hot-start

The AmpliTaq™ DNA Polymerase with Hot-start is provided in an inactive state, using antibody-mediated hot-start mechanism. This allows flexibility in the reaction set-up including premixing of PCR reagents at room temperature. The polymerase is re-activated after a brief hold at 95°C.

When reaction components are combined at room temperature, the inactive enzyme is not capable of primer extension. Any low–stringency mispriming events that may have occurred will not be enzymatically extended and subsequently amplified.

Reverse transcriptase enzyme

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 master mix formulation. To target MMLV, a related virus, or any of the plasmid sequence, it is recommended to design primer sequences not contained in the expression vector. The RT enzyme has been modified to reduce enzyme activity on the benchtop.

dNTPs with dUTP

This master mix includes a blend of dNTPs with dUTP to enable uracil-N-glycosylase (UNG) activity and maintain optimal PCR results. The master mix does not contain uracil-N-glycosylase (UNG), however, the dNTP with dUTP blend in the master mix enables degradation of resulting amplicons, if UNG is added to the reaction.

Recombinant ribonuclease inhibitor

Recombinant ribonuclease inhibitor is a non-competitive inhibitor for ribonucleases. It helps to prevent RNA degredation in the RT-PCR.

ROX™ Passive Reference dye

The ROX™ Passive Reference dye provides an internal reference to which the reporter dye signal can be normalized during data analysis. Normalization is used to correct for fluorescent fluctuations due to changes in concentration or volume.

The master mix is available without ROX™ Passive Reference dye. For more information, see **https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/reagents-kits/other/rox-passive-reference-dye.html**.

C Assay design guidelines

This appendix provides general guidelines to design primers and hydrolysis probes for quantification assays.

Order TaqMan™ Gene Expression Assays

To explore our offerings and order pre-designed TaqMan™ Assays, use our TaqMan™ Assay Search Wizard (**https://www.thermofisher.com/order/taqman-search-wizard/#!/**).

To customize your own TaqMan™ Assay, go to our Assay Design Hub (**https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/assay-design.html**).

To order individual oligos, go to our Custom TaqMan™ Probe webpage (**https://www.thermofisher.com/order/custom-oligo/custom-taqman-probes**).

See the *Custom TaqMan™ Assays Design and Ordering Guide* (Pub. No. 4367671).

Overview of custom assays

The Custom TaqMan™ Assay Design Tool can be used to design custom assays. It can also be used to enter primer and probe sequences.

We recommend the Primer Express™ Software when entering primer and probe sequences in the Custom TaqMan™ Assay Design Tool.

Amplicon site selection

General amplicon site selection guidelines

For access to design tools and additional bioinformatic assay design support, go to the Assay Design Hub: **https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/assay-design.html**.

Using your preferred suite of software tools for sequence analysis and design, select an *amplicon site* within the target sequence. Selecting a good amplicon site ensures amplification of the target without co-amplification of the genomic sequence, pseudogenes, or related genes.

- For gene expression assays, the amplicon should span one or more introns to avoid amplification of the target gene in genomic DNA.
- The shortest amplicons work the best. Consistent results are obtained for amplicon size ranges from 50–150 bp.
- Design the hydrolysis probe before determining primer pairs during assay design.
- Design hydrolysis probes and primer pairs according to the guidelines provided on "Probe and primer design" on page 23.
- The primer pair must be specific to the target; the primer pair must not amplify pseudogenes or other related genes.
- Test the primer pairs, then select the primer pair that produces the highest signal-to-noise ratio (that is, earliest C_t with total RNA or mRNA and no amplification with genomic DNA or negative controls).

If the gene does not contain introns

For gene expression assays, if the gene you are studying does not contain introns, then you cannot ensure amplification of the target cDNA sequence without co-amplification of the genomic sequence.

In this case, you may need to run control reactions that do not contain reverse transcriptase (RT-controls) to determine whether your RNA sample contains DNA. Amplification in the RT-controls indicates that your RNA sample contains DNA. To remove the DNA from the RNA sample, treat the RNA sample with DNase I.

Probe and primer design

Using your preferred suite of software tools for sequence analysis and design, design a probe to detect amplification of the target sequence, then design primers to amplify the target sequence.

This master mix has been optimized for use with primers and hydrolysis probes that have been designed according to our development guidelines. A concentration of 900 nM primers and a 250 nM fluorescent probe generally provides a highly reproducible and sensitive assay.

General probe design guidelines

- Keep the GC content in the 20–80% range.
- Avoid runs of identical nucleotides. If repeats cannot be avoided, there must be fewer than four consecutive G bases.
- The base at the 5’ end must not be a G.
- Select the strand in which the probe contains more C bases than G bases.
- For singleplex assays, keep the T_m between 68–70°C.

General primer design guidelines

- Choose the primers after the probe.
- Do not overlap primer and probe sequences. The optimal primer length is 20 bases.
- Keep the GC content in the 20–80% range.
- Avoid runs of identical nucleotides. If repeats cannot be avoided, there must be fewer than four consecutive G bases.
- Ensure the last 5 nucleotides at the 3' end contain no more than two G and/or C bases.
- If you cannot find acceptable primer sequences, you may need to examine the sequence and select another amplicon site or screen for more sites.

IMPORTANT! Keep the T_m between 58–62°C.

Calculation of oligonucleotide concentrations

After you receive your primers and probe, use a spectrophotometric method to determine the concentrations of the oligonucleotides in your assay.

Calculate oligonucleotide concentrations

1. Calculate the sum of extinction coefficient contributions for each oligonucleotide sequence:

Chromophore	Extinction Coefficient
A	15,200
C	7,050
G	12,010
T	8,400
FAM™ dye	20,958
ABY™ dye	34,500
JUN™ dye	51,600
Cyanine5 dye	10,000
Cyanine5.5 dye	21,500

(continued)

Chromophore	Extinction Coefficient
Alexa Fluor™ 647 dye	23,900
TAMRA™ dye	31,980
TET™ dye	16,255
JOE™ dye	12,000
VIC™ dye	30,100

2. Measure the absorbance at 260 nm (A_{260}) of each oligonucleotide diluted in TE buffer (for example, 1:100).

3. Calculate the oligonucleotide concentration using the following formula:

$$A_{260} = \frac{\text{sum of extinction coefficient contributions} \times \text{cuvette pathlength} \times \text{concentration}}{\text{dilution factor}}$$

Rearrange to solve for concentration:

$$\text{Concentration (C)} = \frac{\text{dilution factor} \times A_{260}}{\text{sum of extinction coefficient contributions} \times \text{cuvette pathlength}}$$

An example calculation of primer concentration

If the primer sequence is `5′-CGTACTCGTTCGTGCTGC-3′`:

- Sum of extinction coefficient contributions:
  = (A × 1) + (C × 6) + (G × 5) + (T × 6)
  = 167,950 $M^{-1}cm^{-1}$
- Example A_{260} measurements:
  Dilution = 1:100
  Cuvette pathlength = 0.3 cm
  A_{260} = 0.13
- Primer concentration:
  = (100 × 0.13) × (167,950 $M^{-1}cm^{-1}$ × 0.3 cm)
  = 2.58×10^{-4} M
  = 258 µM

An example calculation of probe concentration

If the probe sequence is `5′-CGTACTCGTTCGTGCTGC-3′`, FAM™ dye is attached to the 5' end, and TAMRA™ dye is attached to the 3' end:

- Sum of extinction coefficient contributions:
  = (A × 1) + (C × 6) + (G × 5) + (T × 6) + (FAM × 1) + (TAMRA × 1)
  = 220,888 $M^{-1}cm^{-1}$
- Example A_{260} measurements:
  Dilution = 1:100
  Cuvette pathlength = 0.3 cm
  A_{260} = 0.13
- Probe concentration:
  = (100 × 0.13) × (220,888 $M^{-1}cm^{-1}$ × 0.3 cm)
  = 1.96 × 10^{-4} M
  = 196 µM

Guidelines for multiplexing

Multiplex assays can be established for both quantitative and qualitative PCR assays. For relative quantification experiments, multiplexing can be used to determine the relative expression levels between different gene targets within a single sample. Normalization between different samples is achieved by using a reference gene (typically an abundant housekeeping gene). For qualitative presence/absence experiments, multiplexing can be used to determine the presence or absence of specific targets within a sample.

Up to six targets can be multiplexed in a single reaction depending upon the probes that are selected. A number of additional factors can also affect the results of a multiplex assay, including target abundance, and primer and probe concentrations. For both types of multiplex experiments, the goal is to minimize the difference in the C_t values between singleplex and multiplex reactions.

Target abundance

The amount of target (and endogenous control, if used) in a sample can affect the outcome of PCR results when performing multiplex assays. An example of target abundance arranged according to the C_t range for a typical 40-cycle PCR thermal protocol is shown in the following table. Values will differ for different experimental systems, so it is up to the user to determine the actual threshold to use for each expression level.

Target expression level	C_t range
High	$C_t \leq 20$
Medium	$20 < C_t \leq 27$
Low	$27 < C_t \leq 35$
No template control	$C_t > 35$

Different methods are recommended to offset the effects of target abundance when optimizing multiplex assays.

- **Some targets more abundant than others**

  When multiplex PCR is performed on a sample in which one (or more) target(s) is more abundant than the others, the assay(s) for the abundant species should be primer-limited. Typically, housekeeping genes/endogenous controls are high expressors. Using primer-limited reaction conditions prevents consumption of reactants (dNTPs) before the less abundant species begins to amplify.

  Applied Biosystems™ primer-limited assays have final primer concentrations of 150 nM each with 250 nM probe concentration. This is a suggested starting point for customer optimization.

  Note: In addition to limiting primers, for very highly abundant transcripts, probe concentration may need to be adjusted.

- **Targets are of similar abundance**

  In situations where all targets are present in approximately equal abundance, no single assay need be primer-limited. However, assay optimization is recommended to minimize C_t difference between single and multiplex reactions. We recommend starting with 900 nM for each primer and 250 nM for the probe (in the final reaction mix).

- **Either target may be more abundant**

  If any of the targets could be more abundant than the others, depending on the samples being investigated, then all assays need to be primer-limited. Establishing reaction conditions for extreme cases (low/high abundance) is suggested for optimization.

Primer and probe concentration

Optimization of the concentrations of primers and probe for each target is an important first step in assembling a reaction with two or more reporter dyes.

The goal of limiting the primer concentration in the assay is to find the primer concentration that gives the lowest (earliest) possible C_t value for the more abundant target without distorting the C_t value of the less abundant target. Limiting the primer concentration for the more abundant target has the effect of lowering its ΔRn; however, the C_t should remain unchanged under primer-limited conditions. A sample should be assayed using decreasing amounts of primer in order to determine the optimal primer concentration for each assay.

For more information see“Guidelines for multiplexing” on page 26.

Dye selection

It is recommended to make dye/target assignments to balance fluorescence levels in the multiplex reaction.

- FAM™ and ABY™ dyes used with low to medium expressors.
- VIC™ and JUN™ dyes used with medium to high expressors.
- Cyanine5 and Cyanine5.5 dyes with high to medium expressors or controls.

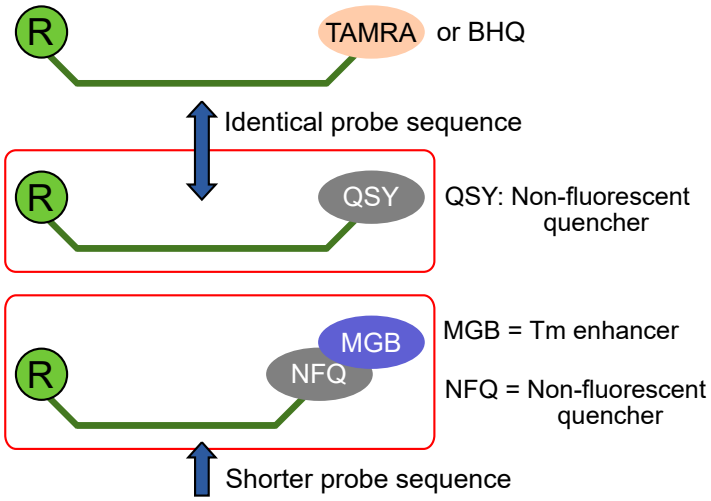
Probe selection

TaqMan™ Assays can use QSY™ probes or MGB probes.

Up to six targets can be multiplexed in a single reaction using QSY™ probes or a mix of QSY™ and MGB probes (FAM™ dye, VIC™ dye, ABY™ dye, JUN™ dye, Cyanine5, and Cyanine5.5).

Note: Use no more than four probes that contain the MGB group.

FAM™ dye and VIC™ dye are available with MGB/NFQ or QSY™ quenchers. For TaqMan™ Gene Expression Assays, order assays with a non-MGB probe at specialty_oligos@thermofisher.com.



Verify singleplex reactions

The first step in a successful multiplex experiment is ensuring that your assays work in singleplex reactions with the dyes and quenchers that you have chosen to use in the multiplex reaction.

1. Prepare concentrated assay mix for each assay according to the expression level of the target. Suggested concentrations are given below. Slight changes may be required for optimal performance.

Target Expression Level	Concentration [1]			
	Assay Mix (Final)	Primer 1	Primer 2	Probe 1
High	20X	3 µM	3 µM	5 µM
Medium	20X	6 µM	6 µM	5 µM
Low	20X	18 µM	18 µM	5 µM

[1] Using a 20X assay mix, the respective concentrations of primers and probes in the reactions will be 150 nM/150 nM/250 nM (High), 300 nM/300 nM/250 nM (Medium), and 900 nM/900 nM/250 nM (Low).

2. Prepare the reaction mixtures according to the table below.

Note: Prepare reaction mixtures based on the number of targets. The TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is optimized for use with multiple assays of up to five targets with the ROX™ passive reference dye

Table 6 Reaction mix preparation for a 20 µL reaction

Component	Singleplex reaction 1	Singleplex reaction 2	Singleplex reaction 3	Singleplex reaction 4	Singleplex reaction 5	Singleplex reaction 6
TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL
FAM™ Assay Mix (20X)	1.0 µL	—	—	—	—	—
VIC™ Assay Mix (20X)	—	1.0 µL	—	—	—	—
ABY™ Assay Mix (20X)	—	—	1.0 µL	—	—	—
JUN™ Assay Mix (20X)	—	—	—	1.0 µL	—	—
Cyanine5 Assay Mix (20X)	—	—	—	—	1.0 µL	—
Cyanine5.5 Assay Mix (20X)	—	—	—	—	—	1.0 µL
Template	≤ 9 µL (1 pg to 100 ng)					
Water	To total volume					
Total	**20 µL**	**20 µL**	**20 µL**	**20 µL**	**20 µL**	**20 µL**

3. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate any air bubbles.

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

5. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly to collect the contents at the bottom of the wells and eliminate any air bubbles.

6. Perform PCR (see “Set up and run the real–time PCR instrument” on page 16).

7. Analyze the results.

Verify the multiplex reaction (see “Verify multiplex reaction” on page 30).

Verify multiplex reaction

After performing verification of singleplex reactions, proceed to evaluation and optimization of the multiplex reaction.

For the appropriate concentrations, see “Verify singleplex reactions” on page 28.

1. Combine verified singleplex concentrations in a multiplex reaction and confirm that they work together.

Component	Multiplex (six-plex) reaction
	96-well Standard plate (20 µL/well)
TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix	5 µL
FAM™ Assay Mix (20X)	1 µL
VIC™ Assay Mix (20X)	1 µL
ABY™ Assay Mix (20X)	1 µL
JUN™ Assay Mix (20X)	1 µL
Cyanine5 Assay Mix (20X)	1 µL
Cyanine5.5 Assay Mix (20X)	1 µL
Template	≤ 9 µL (1 pg to 100 ng)
Water	To total volume
Total	**20 µL**

2. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate any air bubbles.
3. Transfer the appropriate volume of each reaction to each well of an optical plate.
4. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly collect the contents at the bottom of the wells and eliminate any air bubbles.
5. Perform PCR (see “Set up and run the real–time PCR instrument” on page 16).

Evaluate PCR results

Evaluate the real-time PCR results to verify that the reaction efficiency, ΔC_t between singleplex and multiplex reactions, and standard deviation of replicates are not compromised using the selected multiplex conditions. Ideally there should be no difference between the results from single and multiplex reactions under the selected conditions.

- Reaction efficiency

  The standard curve method is recommended to evaluate (optimize) multiplex assays. Run as many 10-fold dilution points, in triplicate, as possible for the sample(s) and assays being investigated for each assay singly and in multiplex. A minimum of 3 logs should be used, but up to 6 logarithmic units (seven 10-fold dilution points) is ideal. Ensure that the dynamic range of the standard curve

is broad enough to encompass most of the experimental samples, bearing in mind that the expression levels of the target(s) of interest may vary widely between samples.

Take a careful look at the standard curve to verify that there is a good fit of the line to all the dilution points, and that the correlation coefficient (R^2) of the line is 0.98 or higher. A lower R^2 value indicates that some of the dilutions (usually the lowest, highest, or both) do not fall within the range of the standard curve. For more information, go to **thermofisher.com/qpcreducation**.

Results are analyzed in a plot of *log*[template amount]$_{(x\text{-axis})}$ against C_t value$_{(y\text{-axis})}$

The slope of the line is used to calculate the PCR efficiency using the formula:

Efficiency = $10^{(-1/\text{slope})}$ - 1

The target efficiency for 5 to 6 logarithmic units should be 100% +/- 10% in both singleplex and multiplex reactions. If there are significant differences, re-optimize the primer and probe concentrations.

- ΔC_t between singleplex and multiplex reactions

  Using the dilution series, calculate ΔC_t value between the target in singleplex and multiplex reactions. The ΔC_t values between multiplex and singleplex reactions should be as close as possible (e.g., $\Delta C_t \leq 1$).

  Differences in C_t between single and multiplex reactions can often be mitigated by adjusting primer concentrations. Adjustments following the general guidelines provided under may be required (see “Verify singleplex reactions” on page 28).

- Standard deviation

  A high standard deviation of the C_t for the sample replicates indicates that other factors, such as competition or inhibition in the multiplex reaction, are contributing to the lack of reproducibility.

  Determine the standard deviations of samples assayed as single and multiplex reactions. High standard deviations of C_t values in multiplex reactions can often be minimized by adjustments to the cycling conditions. Increasing the anneal/extend times (to 30 to 45 seconds) is suggested if the standard deviations in multiplex reactions increase relative to singleplex.

Experimental design guidelines

User-defined assays

To design your own assay for use with the master mix, see *TaqMan™ Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189) and **https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/assay-design.html**.

Note: The term *assay* refers to the primer and probe set.

Experiment types

The master mix is compatible with the following types of experiments.

- **Presence/absence**
  - An endpoint experiment that indicates the presence or absence of a specific nucleic acid sequence (target) in a sample.
  - The actual quantity of target is not determined.
  - Presence/absence experiments are commonly used to detect the presence or absence of viral or bacterial pathogens. (Presence/absence experiments are also called *plus/minus experiments*.)
- **Standard curve**
  - A type of quantification experiment that determines the absolute target quantity in samples.
  - With the standard curve method, the real-time PCR system software measures amplification of the target in samples and in a standard dilution series.
  - Data from the standard dilution series are used to generate the standard curve.
  - Using the standard curve, the software interpolates the absolute quantity of target in the samples.

  - Standard curve experiments are commonly used for quantifying viral load. (Standard curve experiments are also called *absolute quantification* or *AQ experiments*.)
  - To collect only the C_t (C_q) values, perform a standard curve experiment without running standards.
- **Relative quantification**
  - A type of quantification experiment that compares changes in gene expression in a given sample relative to another reference sample, such as an untreated control sample.
  - Relative quantification (RQ) can be performed with data from all real–time PCR instruments.
  - Relative quantification uses the standard curve and comparative C_t calculation methods.
  - Relative quantification does not allow single–sample results to be meaningful, nor does it allow gene–to–gene quantitative comparisons. It is used for sample–to–sample quantitative comparisons.

Note: A quantification experiment is a real-time experiment that measures the quantity of a target nucleic acid sequence (target) during each amplification cycle of the polymerase chain reaction (PCR).

Guidelines for real-time PCR

Item	Guideline
Assays (primer and probe set)	Keep all assays in the freezer, protected from light, until you are ready to use them. Excessive exposure to light might affect the fluorescent probes.
	Just before use, allow the assays to thaw on ice.
	At initial use, aliquot the assays to avoid multiple freeze/thaw cycles.
TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix	Store the master mix in the freezer, protected from light, until you are ready to use it.
	Just before use, remove the master mix from the freezer and place on ice or on the benchtop. When in use, the master mix may be placed on the benchtop or on ice. **Note:** The master mix does not freeze at recommended storage conditions. Under colder storage conditions, the master mix might appear unfrozen, partially frozen, crystallized, or semi-solid. Place the master mix on the benchtop or on ice to return to its liquid state. While in use, the master mix can be stored at room temperature (15–24°C) for up to 1 week or at 2–8°C for up to 1 month.
(For standard curve experiments) Standards	Standards are critical for accurate analysis of run data. Mistakes or inaccuracies in making the dilutions directly affect the quality of the results. The quality of pipettes and tips and the care used in measuring and mixing dilutions affect accuracy. Use TE buffer or RT-qPCR Grade Water to prepare the standard dilution series.

(continued)

Item	Guideline
Thermal-cycling temperature ranges	The optimal temperatures for reverse transcription and annealing are recommended in this protocol (see step 2 on page 16). However, in some instances you may wish to alter the temperatures. • The RT enzyme is optimal at 53°C and can operate in the range of 48–55°C. • Be sure the annealing temperature is consistent with the melting temperature (T_m) of your primer designs. For guidelines on designing primers, see "Probe and primer design" on page 23. **Note:** *(Optional)*With some assays, the addition of a 10 minute 85°C incubation step between the reverse transcription and activation steps can improve replicate consistency.
Multiplexing	TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix is designed to accommodate running multiple assays simultaneously. For guidelines on designing multiplex reactions, see *TaqMan™ Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189).

Recommended types of reactions

For each experiment type, the following types of reactions are needed.

Experiment type	Reaction type	Description
Presence/absence	Unknown	A well that contains: • Sample (DNA or RNA in which the presence of a target is unknown) • TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix • Assay of choice
	Exogenous Internal positive control (IPC)	A short synthetic DNA or RNA template that you can add to the reactions to distinguish between true negative results and reactions affected by PCR inhibitors, incorrect assay setup, or a reagent or instrument failure.
	No amplification control (NAC)	A well that contains all reaction components except the unknown sample and IPC. Alternatively, the well may contain the IPC plus a blocking agent for the IPC. No amplification should occur in NAC wells.
	No template control (NTC)	A well that contains all PCR components except the unknown sample. Only the IPC should amplify in NTC wells.
	Replicate	A well that is identical to another. The wells contain identical components and volumes. Performing at least three replicates of each reaction is recommended.

(continued)

Experiment type	Reaction type	Description
Relative quantification	Unknown	A well that contains: • Sample (DNA or RNA for which the quantity of a target is unknown) • TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix • Assay of choice • Assay for reference gene
	No template control (NTC)	A well that contains all PCR components except the unknown sample. Only the IPC, if used, should amplify in NTC wells.
	Replicate	A well that is identical to another. The wells contain identical components and volumes. Performing at least three replicates of each reaction is recommended.
Standard curve	Unknown	A well that contains: • Sample (DNA or RNA in which the quantity of the target is unknown) • TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix • Assay of choice
	Standard	A well that contains DNA or RNA of a known standard quantity; used in quantification experiments to generate standard curves. **Note:** You can perform a standard curve experiment without running standards, if you only want to collect the C_t values.
	Standard dilution series	A set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards.
	No template control (NTC)	A negative control well that contains water or buffer instead of sample. No amplification of the target should occur in negative control wells.
	Replicate	A well that is identical to another. The wells contain identical components and volumes. Performing at least three replicates of each reaction is recommended.

E Recommended 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 other DNA decontamination solution, and RNase decontamination solution (see “Required materials not supplied” on page 9).

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.

F

Safety

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

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

Chemical safety

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

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

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

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

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

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

G

Definition of symbols

The following table describes symbols that are present on product labels or product documents. Symbols conform to applicable international regulatory standards.

Symbol	Description
	MANUFACTURER
	COUNTRY OF ORIGIN
	USE BY
	BATCH CODE
	CATALOG NUMBER
	UPPER AND LOWER LIMITS OF TEMPERATURE
	PROTECT FROM LIGHT
	CONSULT INSTRUCTIONS FOR USE
	READ SAFETY DATA SHEET (SDS)
	UNIQUE DEVICE IDENTIFIER

(continued)

Symbol	Description
	CONTAINS BIOLOGICAL MATERIAL OF ANIMAL ORIGIN

Documentation and support

Related documentation

Document	Pub. No.
TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix Quick Reference	MAN0028388
TaqMan™ Multiplex PCR Optimization User Guide	MAN0010189
Custom TaqMan™ Assays Design and Ordering Guide	4367671

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**.