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

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

Pub. No. MAN0028388 Rev. A.0

Contents and storage

Table 1 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 2 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.

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

- 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.

General guidelines

Prevent contamination

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

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.

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.

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

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.
RNA or DNA sample	≤14 µL	≤154 µL	Use as much sample as required, up to 70% of the total volume. [4]
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] 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.

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.

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**.

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
	CONTAINS BIOLOGICAL MATERIAL OF ANIMAL ORIGIN

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Revision	Date	Description
A.0	2 January 2024	New document for the TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix.

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