TaqPath™ 1-Step Multiplex Master Mix

Catalog Numbers A28521, A28522, A28523, A28525, A28526, and A28527

Pub. No. MAN0014389 **Rev.** B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqPath™ 1-Step Multiplex Master Mix User Guide* (Pub. No. MAN0014269). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Contents and storage

Product	Cat. No.	Amount	Storage
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28521	1 × 0.5 mL	–30°C to –10°C
	A28522	5 × 1 mL	
	A28523	1 × 10 mL	
TaqPath™ 1-Step Multiplex Master Mix	A28525	1 × 0.5 mL	
	A28526	5 × 1 mL	
	A28527	1 × 10 mL	

General guidelines

This quick reference provides simplified instructions for using the TaqPath™ 1-Step Multiplex Master Mix and the TaqPath™ 1-Step Multiplex Master Mix (No ROX™) for performing gene expression assays or detecting RNA virus targets.

The TaqPath™ 1-Step Multiplex Master Mix contains MUSTANG PURPLE™ dye as a passive reference dye (absorption 647 nm, emission 654 nm).

The TaqPath™ 1-Step Multiplex Master Mix (No ROX™) does not contain a passive reference dye.

For detailed instructions and ordering information for additional products, see the *TaqPath™ 1-Step Multiplex Master Mix User Guide* (Pub. No. MAN0014269).

- Mix the master mix thoroughly to ensure that the solution is homogenous before use.
- If preparing a reaction mix, scale all components except the template according to the number of reactions to be performed. Include 10% overage to account for variations in pipetting.
- For Fast real-time PCR systems, use 20 µL reaction volumes for each well.
- For Standard real-time PCR systems, use 50 µL reaction volumes for each well.

Prepare the reaction mix

1. Thaw all reagents on ice.
2. Calculate the total volume required for each reaction component according to the following table.

Component	Fast systems (20–µL reaction)	Standard systems (50–µL reaction)	Notes
TaqPath™ 1-Step Multiplex Master Mix (4X)	5 µL	12.5 µL	—
Up to four user-defined assays (primers and probe)[1]	1 µL/assay	2.5 µL/assay	Use primer concentrations of 150–900 nM and a probe concentration of 100–250 nM.
RNA Sample	Variable	Variable	Use as much sample as needed, up to the maximum allowed by the reaction volume.
RT-PCR Grade Water	Variable	Variable	Fill to the total reaction volume.
Total volume per reaction	**20 µL**	**50 µL**	**—**

[1] Potential assays include the TaqMan™ Assay Mix, FAM™ dye (20X); TaqMan™ Assay Mix, VIC™ dye (20X); TaqMan™ Assay Mix, ABY™ dye (20X); and TaqMan™ Assay Mix, JUN™ dye (20X).

3. Working on ice, add the components directly to each well of an optical reaction plate.
4. Cover 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™ 1-Step Multiplex Master Mix is a 4X formulation and is more viscous than most master mixes. Ensure that all of the components are thoroughly mixed in all the wells before proceeding. It has been observed that inverting the plate gives more uniform mixing across the reaction plate than vortexing.

5. Centrifuge the plate at 150 × *g* (1000 rpm) for 1 minute to collect the contents at the bottom of the wells and eliminate air bubbles.

Run the RT-real-time PCR plate

Run the plate on an Applied Biosystems™ real-time PCR instrument. See the appropriate instrument user guide for detailed instructions to program the thermal cycling conditions or to run the plate.

1. In the real-time PCR system software, open the plate document or experiment that corresponds to the reaction plate.

   See the following table for the thermal cycling conditions.

Step	Stage	Cycles	Temperature	Time for Fast systems	Time for Standard systems
UNG incubation	1	1	25°C	2 minutes	2 minutes
Reverse transcription[1]	2	1	53°C	10 minutes	10 minutes
Polymerase activation[2]	3	1	95°C	2 minutes	2 minutes
Amplification	4	40	95°C	3 seconds	15 seconds
			60°C	30 seconds	1 minute

[1] Reverse transcription works best between 48°C and 55°C.
[2] Required for RT inactivation, initial denaturation, and to activate the DNA polymerase.

2. Verify the appropriate reaction volume is selected for your experiment.
3. Select the passive reference dye.

Option	Description
MUSTANG PURPLE™	TaqPath™ 1-Step Multiplex Master Mix
None	TaqPath™ 1-Step Multiplex Master Mix (No ROX™)

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

5. Start the run.

Guidelines for analysis

1. View the amplification plot, and modify as needed.
   - Set the baseline and threshold values.
   - Remove outliers from the analysis.

2. In the well table or results table, view the C_t values for each well and for each replicate group.

3. *(For standard curve experiments)* View the standard curve for the following items:
   - Slope
   - Amplification efficiency
   - R^2 values
   - Y-intercept
   - C_t values
   - Outliers

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

Revision history: Pub. No. MAN0014389

Revision	Date	Description
B.0	30 October 2019	• Updated for general style and formatting. • Added thermal cycling conditions for standard reactions. • Corrected recommended primer concentrations for RT-real-time PCR reaction mix.
A.0	8 February 2016	New document.