

VetMAX™ Fast Multiplex Master Mix (with ROX)

Catalog Numbers A57081, A57305, A57306

Pub. No. MAN0028688 Rev. C

Note: For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: *TaqMan™ Fast Virus 1-Step Master Mix User Guide* (Pub. No. MAN0028278). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

VetMAX™ Fast Multiplex Master Mix (with ROX) is designed to be a fast-cycling-capable, multiplex-assay-compatible one-step RT-PCR master mix for testing of animal sourced samples. It can be used to amplify up to four DNA or RNA targets present in a variety of animal sample matrices, including those with challenging PCR inhibitors. The fast-cycling capability, combined with multiplexing compatibility, enables rapid and accurate real-time PCR results in under 50 minutes.

Contents and storage

Cat. No.	Contents	Number of 20-µL reactions	Storage ^[1]
A57081	1 × 1 mL	100	-25°C to -15°C
A57305	5 × 1 mL	500	
A57306	1 × 10 mL	1000	

^[1] See packaging for expiration date.

Set up the RT-PCR reaction

1. Thaw the reagents and nucleic acid samples on ice. Resuspend the nucleic acid samples by inverting the tube, then gently vortexing.
2. Mix the VetMAX™ Fast Multiplex Master Mix thoroughly but gently until homogenous. Make sure it is fully thawed and not viscous.
3. Prepare the RT-PCR Reaction Mix for the number of reactions required as shown in table below, plus 10% overage.

Component	96- or 384-well PCR Plate
VetMAX™ Fast Multiplex Master Mix (2X)	10 µL
20X TaqMan™ Primer/Probe Mix ^[1]	1 µL
Sample RNA/DNA or Nuclease Free Water	9 µL
Total volume	20 µL

^[1] A primer concentrations of 400–900 nM and a probe concentration of 100–250 nM is recommend.

4. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
5. Seal the plate with an optical adhesive cover, then vortex briefly or invert the plate to mix the contents.
6. 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 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. Select the appropriate cycling mode.

The master mix is compatible with Fast or Standard cycling modes.

Note: The cycling mode depends solely on the type of master mix used in the reaction, and not the plate format.

2. Set up the thermal cycler protocol.

Table 1 Fast cycling mode

Step	Temperature	Time	Cycles
Reverse transcription ^[1]	50°C	5 minutes	1
Taq activation	95°C	10 minutes	1
Denaturation	95°C	3 seconds	40
Annealing / extension ^[2]	60°C	30 seconds	

^[1] RT enzyme will function best in the range of 48°C to 55°C..

^[2] Ensure that the annealing temperature is consistent with the melting temperature (T_m) of the primers used in the reaction.

Table 2 Standard cycling mode

Step	Temperature	Time	Cycles
Reverse transcription ^[1]	50°C	5 minutes	1
Taq activation	95°C	10 minutes	1
Denaturation	95°C	15 seconds	40
Annealing / extension ^[2]	60°C	60 seconds	

^[1] RT enzyme will function best in the range of 48°C to 55°C..

^[2] Ensure that the annealing temperature is consistent with the melting temperature (T_m) of the primers used in the reaction.

3. Select the appropriate assay reporter dyes and indicate **ROX** as the **Passive Reference Dye**.
4. Load the plate into the real-time PCR instrument.
5. Start the run.

Analyze the data

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

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

Revision history: Pub. No. MAN0028688 C

Revision	Date	Description
C	24 June 2025	The regulatory statement was updated.
B00	19 April 2024	The manufacturing address was updated to Austin, TX, USA.
A.0	16 March 2023	Initial release for VetMAX™ Fast Multiplex Master Mix (with ROX).

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

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