

## 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe

### Product Name and Catalog Number

1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe, Cat RT-PCR 149, 2x1mL/5x1mL/10mL for 200/500/1000 reactions x 20µL (No ROX, Low ROX or High ROX)

### Intended Use

- The 1-Step 2X Super Multiplex RT-PCR Master Mix is used for real-time qualitative and quantitative multiplex RT-PCR amplifications with TaqMan probes for up to four targets.
- The master mix is a premixed, 2X concentrated solution that has all the components except for gene-specific primers, probes and RNA templates.

### Kit Characteristics

- The kit is designed for multiplex RT-PCR with TaqMan probes.
- For the reverse transcription step, this kit uses a highly efficient Thermophilic Reverse Transcriptase (US patent pending), which is a thermophilic type A polymerase, with optimal temperatures of 60-62°C.
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The RTase efficiently synthesizes complementary DNA strands on RNA templates from gene-specific primers, ≥1 unit per 20µL of reaction.
- The RTase reversely-transcribes single digit copies of target RNA molecules consistently.
- The kit also contains Super Taq-Probe DNA polymerase, specially engineered for TaqMan probes and generating S-shaped curves.
- Up to four pairs of gene-specific primers can be applied in one reaction.
- The concentrations of the primers and probes are variable depending on assay designs and thermocycling protocols (Table 1).
- The preferable PCR product size is ≤150bp.
- The kit has three formulations of ROX, Low ROX or High ROX concentration for your choice.

### Kit Contents

2X Master Mix (2x1mL/5x1mL/10mL for 200/500/1000 reactions x 20µL) and an instruction for use.

### Transportation and Storage

The kit can be transported at below 4°C for up to 3 days. The kit should be kept stable in the dark at -20°C for ≤24 months with ≤10 times of freeze-thaw cycles. The kit can be stored at 4°C for a week.

### Setup Reaction and Thermocycling

- Thaw 1-Step 2X RT-PCR Master Mix and other reaction components at room temperature, mix each component, centrifuge and then place on ice.
- Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge and then place on ice.
- Aliquot the assay mix into PCR tubes or plate.
- Add RNA template to PCR tubes or plate.
- Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.
- Mix and then briefly centrifuge the tubes or plate.
- Program PCR instrument with indicated thermo-cycling protocol.
- Load PCR tubes or plate and start to run.
- Perform data analysis according to the PCR instrument instructions.

**Table 1. Setting up a 20µL or 10µL reaction**

Component	Volume per 20µL	Volume per 10µL	Final concentration
2X Master Mix	10µL	5µL	1X
Primers <sup>a</sup>	Variable	Variable	Each 150-900nM
TaqMan probes <sup>b</sup>	Variable	Variable	Each 150-250nM
RNA templates <sup>c</sup>	Variable	Variable	As low as single digit copies of target RNA to ≤1µg total RNA
H <sub>2</sub> O	To 20µL	To 10µL	

### Footnotes of Table 1

<sup>a</sup>The primer's T<sub>m</sub> should be designed ≥60°C, preferably between 62°C to 65°C, using primer3 software for high efficiency and specificity.

<sup>b</sup>The probe's T<sub>m</sub> should be 8-10°C higher than the primer's T<sub>m</sub>, preferably between 70-75°C.

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RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

**Table 2. Compatible instruments**

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

**Table 3. Standard thermocycling protocol**

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	

**Table 4. Three-Step Thermocycling Protocol**

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal	30sec	

	acquisition		
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**Footnotes of Tables 3 and 4**

The three-step thermocycling protocol in Table 4 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 3.

The primer concentration used in Tables 6 and 7 is typically 0.15-0.2uM.

**Related Products**

- Thermophilic Reverse Transcriptase, Cat RT-PCR 140
- Taq-Probe Polymerase, Cat RT-PCR 145
- Taq-Fast Polymerase, Cat RT-PCR 148
- 1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat RT-PCR 147
- 1-Step 2X RT-PCR Master Mix-TaqMan Probe, Cat RT-PCR 143
- 1-Step 2X Multiplex RT-PCR Master Mix-TaqMan Probe, Cat RT-PCR 146
- 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe, Cat RT-PCR 149

**Precautions**

If you order a "No ROX" master mix but have an Applied Biosystems/ThermoFisher instrument, please **turn off ROX passive reference dye button** when setup assays.