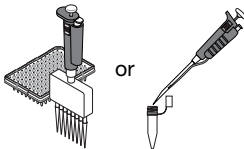


# TaqMan<sup>®</sup> RNA-to-C<sub>T</sub><sup>™</sup> 1-Step Kit

For safety and biohazard guidelines refer to the “Safety” section in the *TaqMan<sup>®</sup> RNA-to-C<sub>T</sub><sup>™</sup> 1-Step Kit Protocol* (PN 4393463). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card provides abbreviated procedures. For complete procedures, refer to the *TaqMan<sup>®</sup> RNA-to-C<sub>T</sub><sup>™</sup> 1-Step Kit Protocol* (PN 4393463).

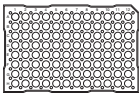
## 1 Prepare the RT-PCR reactions.



Component (Applied Biosystems Gene Expression Assays)	Volume for One Reaction		
	10 µL	20 µL	50 µL
<b>TaqMan<sup>®</sup> RT-PCR Mix (2X)</b>	5.0 µL	10.0 µL	25.0 µL
TaqMan <sup>®</sup> Gene Expression Assay (20X)	0.5 µL	1.0 µL	2.5 µL
TaqMan <sup>®</sup> RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (up to 1 µg) + RNase-free H <sub>2</sub> O	4.25 µL	8.5 µL	21.25 µL
<b>Total Volume</b>	<b>10.0 µL</b>	<b>20.0 µL</b>	<b>50.0 µL</b>

Component (Custom-Designed Gene Expression Assays)	Volume for One Reaction		
	10 µL	20 µL	50 µL
<b>TaqMan<sup>®</sup> RT-PCR Mix (2X)</b>	5.0 µL	10.0 µL	25.0 µL
Forward primer (900 nM final)	Variable	Variable	Variable
Reverse primer (900 nM final)	Variable	Variable	Variable
TaqMan probe (50 to 250 nM final)	Variable	Variable	Variable
TaqMan <sup>®</sup> RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (up to 1 µg)	Variable	Variable	Variable
RNase-free H <sub>2</sub> O	to 10 µL	to 20 µL	to 50 µL
<b>Total Volume</b>	<b>10.0 µL</b>	<b>20.0 µL</b>	<b>50.0 µL</b>

## 2 Prepare the reaction plate.



Use a reaction plate appropriate for your real-time PCR system:

- MicroAmp<sup>™</sup> Fast Optical 48-Well Reaction Plate: 20 µL
- MicroAmp<sup>™</sup> Fast Optical 96-Well Reaction Plate: 20 µL
- MicroAmp<sup>™</sup> Optical 96-Well Reaction Plate: 50 µL
- MicroAmp<sup>™</sup> Optical 384-Well Reaction Plate: 10 µL

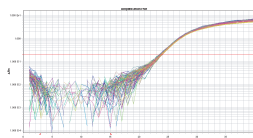
### 3 Run the RT-PCR reaction plate.



- Ramp speed or mode: **Standard**
- Reaction volume (µL): **10, 20, or 50**
- Thermal cycling conditions:

Stage	Temp	Time
Holding	48 °C	15 min
Holding	95 °C	10 min
Cycling (40 cycles)	95 °C	15 sec
	60 °C	1 min

### 4 Analyze the experiment.



- **Standard curve** (standard curve and relative standard curve experiments) – Slope, amplification efficiency, R<sup>2</sup> values, y-intercept, C<sub>T</sub> values, outliers
- **Gene expression plot** (relative standard curve and comparative C<sub>T</sub> experiments) – Differences in gene expression, standard deviation in the replicate groups
- **Amplification plots** – Baseline and threshold values, outliers
- **Well table or results table** – C<sub>T</sub> values for each well and for each replicate group

## TaqMan® RNA-to-C<sub>T</sub>™ 1-Step Kit Products

Quantity	Part Number
Reagents sufficient for 40 × 50-µL reactions: <ul style="list-style-type: none"> <li>• TaqMan® RT-PCR Mix (2X), 1 mL</li> <li>• TaqMan® RT Enzyme Mix (40X), 50 µL</li> </ul>	4392653
Reagents sufficient for 200 × 50-µL reactions: <ul style="list-style-type: none"> <li>• TaqMan® RT-PCR Mix (2X), 5 mL</li> <li>• TaqMan® RT Enzyme Mix (40X), 250 µL</li> </ul>	4392938
Reagents sufficient for 2000 × 50-µL reactions: <ul style="list-style-type: none"> <li>• TaqMan® RT-PCR Mix (2X), 10 × 5 mL</li> <li>• TaqMan® RT Enzyme Mix (40X), 10 × 250 µL</li> </ul>	4392656

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**NOTICE TO PURCHASER:** PLEASE REFER TO THE *TaqMan® RNA-to-C<sub>T</sub>™ 1-Step Kit Protocol* (PN 4393463) FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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12/2007

