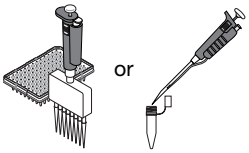
Applied Biosystems

Power SYBR® Green RNA-to-C_T™ *1-Step* Kit

For safety and biohazard guidelines refer to the "Safety" section in the *Power SYBR® Green RNA-to-C_T™ 1-Step Kit Protocol* (PN 4391003). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

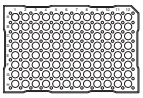
This quick reference card provides abbreviated procedures. For complete procedures, refer to the *Power SYBR® Green RNA-to-C_T™ 1-Step Kit Protocol* (PN 4391003).

1 Prepare the RT-PCR reactions.



Component	Volume for One Reaction		
	10 µL	**20 µL**	**50 µL**
***Power* SYBR® Green RT-PCR Mix (2×)**	5.0 µL	10.0 µL	25.0 µL
Forward primer (100 to 200 nM final)	Variable	Variable	Variable
Reverse primer (100 to 200 nM final)	Variable	Variable	Variable
RT Enzyme Mix (125×)	0.08 µL	0.16 µL	0.4 µL
RNA template (up to 100 ng)	Variable	Variable	Variable
RNase-free H_2O	to 10 µL	to 20 µL	to 50 µL
Total Volume	**10.0 µL**	**20.0 µL**	**50.0 µL**

2 Prepare the reaction plate.



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

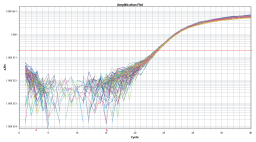
- MicroAmp™ Fast Optical 48-Well Reaction Plate: 20 µL
- MicroAmp™ Fast Optical 96-Well Reaction Plate: 20 µL
- MicroAmp™ Optical 96-Well Reaction Plate: 50 µL
- MicroAmp™ Optical 384-Well Reaction Plate: 10 µL

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	30 min
Holding	95 °C	10 min
Cycling (40 cycles)	95 °C	15 sec
	60 °C	1 min
Melt Curve (optional)	95 °C	15 sec
	60 °C	15 sec
	95 °C	15 sec

4 Analyze the experiment.



- **Standard curve** (standard curve and relative standard curve experiments) – Slope, amplification efficiency, R^2 values, y-intercept, C_T values, outliers
- **Gene expression plot** (relative standard curve and comparative C_T experiments) – Differences in gene expression, standard deviation in the replicate groups
- **Amplification plots** – Baseline and threshold values, outliers
- **Well table or results table** – C_T values for each well and for each replicate group
- *(Optional)* **Melt curve** – Number of T_m peaks

Power SYBR® Green RNA-to-C_T™ *1-Step* Kit Products

Quantity	Part Number
Reagents sufficient for 40 × 50-µL reactions: • *Power* SYBR® Green RT-PCR Mix (2X), 1 mL • RT Enzyme Mix (125X), 20 µL	4391178
Reagents sufficient for 200 × 50-µL reactions: • *Power* SYBR® Green RT-PCR Mix (2X), 5 mL • RT Enzyme Mix (125X), 80 µL	4389986

12/2007

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