

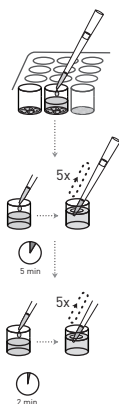
Power SYBR® Green Cells-to-Ct™ Kit

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Note: For safety and biohazard guidelines, refer to the “Safety Information” appendix in the *Power SYBR® Green Cells-to-Ct™ Kit Protocol* (P/N 4403787). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference Card is designed as a benchtop reference for experienced users. Read the *Power SYBR® Green Cells-to-Ct™ Kit Protocol* (PN 4403786) before using the kit for the first time.

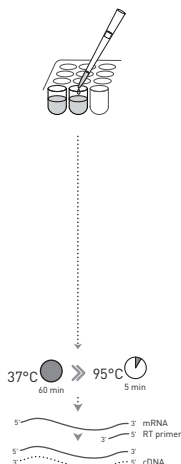
1 Cell Lysis



- a. Wash cells in cold PBS in the culture plate, or wash and transfer $\leq 10^5$ cells to each tube or well for lysis.
- b. (Optional) Dilute DNase I 1:100 in Lysis Solution for step c.
- c. Add 50 μ L Lysis Solution and mix 5 times by pipetting up and down.
- d. Incubate for 5 min at room temp (19–25 °C).
- e. Add 5 μ L Stop Solution (6 μ L Stop Solution with Xeno™ RNA Control) and mix 5 times by pipetting up and down.
- f. Incubate for 2 min at room temp. Do not incubate longer than 20 min at room temp.

STOPPING POINT Potential.

2 Reverse Transcription (RT)



- a. Program the thermal cycler for the RT: 60 min at 37 °C; 5 min at 95 °C; hold at 4 °C.
- b. Assemble an RT Master Mix and distribute it to reaction tubes/plates.

Component	Each rxn	96 rxns
2X SYBR® RT Buffer	25 μ L	2.64 μ L
20X RT Enzyme Mix†	2.5 μ L	264 μ L
Nuclease-free Water	12.5 μ L	1.32 μ L
Final volume RT master mix	40 μL	4.22 μL

† For the minus-RT control, use Nuclease-free Water in place of 20X RT Enzyme Mix.

- c. Add lysate and mix thoroughly (10 μ L lysate).
- d. Run the RT thermal cycler program.

STOPPING POINT Potential.



3 Real-Time PCR

a. Program the real-time PCR instrument.

	Stage	Reps	Temp	Time
Enzyme Activation (hold)	1	1	95 °C	10 min
PCR (cycle)	2	40	95 °C	15 sec
			60 °C	1 min
Dissociation Curve	3	(use default setting)		

b. Assemble PCR Cocktail and distribute to reaction tubes/plates.

Component	20 µL PCRs Each rxn	50 µL PCRs Each rxn
Power SYBR® Green PCR Master Mix	10 µL	25 µL
Forward & Reverse PCR Primers [†]	variable	variable
Nuclease-free water	variable	variable
Final volume PCR cocktail	16 µL	40 µL

[†] Generally a 200–400 nM final concentration of each PCR primer provides good results. For instructions on optimizing PCR primer concentrations, see the *Applied Biosystems Power SYBR® Green PCR Master Mix and RT-PCR Protocol*, P/N 4367218.

c. Add cDNA samples and mix thoroughly.

Component	20 µL PCRs	50 µL PCRs
PCR Cocktail	16 µL	40 µL
RT Reaction (cDNA)	4 µL	10 µL

d. Run the PCRs in a real-time PCR instrument.



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