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Fast SYBR® Green Cells-to-CT™ Kit

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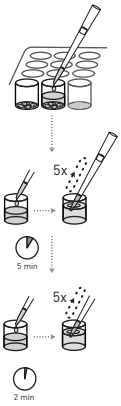
Note: For safety and biohazard guidelines, refer to the "Safety Information" appendix in the *Fast SYBR® Green Cells-to-Ct™ Kit Protocol (P/N 4403786)* . For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference Card is designed as a benchtop reference for experienced users. Read the *Fast SYBR® Green Cells-to-Ct™ Kit Protocol* (P/N 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 ≤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 µL Lysis Solution and mix 5 times by pipetting up and down.

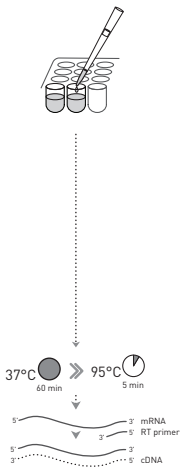
d. Incubate for 5 min at room temp (19–25 °C).

e. Add 5 µL Stop Solution (6 µL 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
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2X SYBR® RT Buffer	25 µL	2.64 µL
20X RT Enzyme Mix†	2.5 µL	264 µL
Nuclease-free Water	12.5 µL	1.32 µL
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 µL lysate).

d. Run the RT thermal cycler program.

STOPPING POINT Potential.

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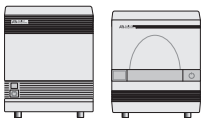
3 Fast Real-Time PCR



a. Program the real-time PCR instrument.

	Stage	Reps	Temp	Time
Enzyme Activation (hold)	1	1	95 °C	20 sec
PCR (cycle)	2	40	95 °C	3 sec
			60 °C	30 sec
Dissociation Curve	3	(use default setting)		

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

Component	20 µL PCRs Each rxn
Fast SYBR® Green PCR Master Mix	10 µL
Forward & Reverse PCR Primers†	variable
Nuclease-free water	variable
Final volume PCR cocktail	16 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 Fast SYBR® Green PCR Master Mix Protocol*, P/N 4385372..

c. Add cDNA samples and mix thoroughly.

Component	Volume
PCR Cocktail	16 µL
RT Reaction (cDNA)	4 µL

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

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