

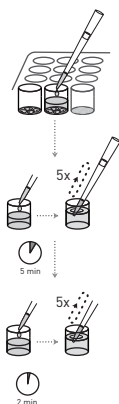
Fast SYBR® Green Cells-to-Ct™ Kit

Publication Part Number 4405659 Rev. B Revision Date March 2011

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.

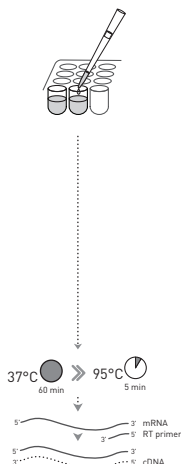
1 Cell Lysis



- a. Wash cells in cold PBS in the culture plate, or wash and transfer $\leq 10^5$ 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
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 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.

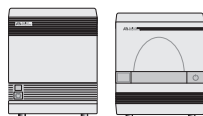
Component	20 µL PCRs Each rxn
Fast SYBR® Green PCR Master Mix	10 µL
Forward & Reverse PCR Primers [†]	variable
Nuclease-free water	variable
Final volume PCR cocktail	16 µL

[†] 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.



For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

LIMITED USE LABEL LICENSE: RESEARCH USE ONLY

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Human diagnostic uses require a separate license from Roche.

© 2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

