SYBR™ Green Fast Advanced Cells-to-C_T™ Kit

Catalog Numbers A35379, A35380, A35381

Pub. No. MAN0017756 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *SYBR™ Green Fast Advanced Cells-to-C_T™ Kit User Guide* (Pub. no. MAN0017755). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users of the SYBR™ Green Fast Advanced Cells-to-C_T™ Kit (Cat. No. A35379). Refer to the *SYBR™ Green Fast Advanced Cells-to-C_T™ Kit User Guide* (Pub. No. MAN0017755) for detailed instructions.

Before you begin

- Thaw the Stop Solution and mix thoroughly by inverting or flicking several times, then place on ice.

IMPORTANT! Do not vortex.

- Chill 1X PBS to 4°C.
- *(Optional)* Add DNase I (1:100) to Lysis Solution, to remove genomic DNA during cell lysis, according to the following table.

Component	Volume		
	per reaction	96 reactions[1]	384 reactions
Lysis Solution	49.5 µL	5.23 mL	20.91 mL
DNase I	0.5 µL	52.8 µL	211 µL
Total	**50 µL**	**5.28 mL**	**21.12 mL**

[1] Includes 10% overage

- *(Optional)* To include an exogenous control using the SYBR™ Green Cells-to-C_T™ Control Kit, add 1 µL of Xeno™ RNA Control per 5 µL of Stop Solution.

Prepare cells for lysis

1 Prepare cells for lysis

Prepare adherent or suspension cells for lysis.

1. Add 50 µL of 4°C 1X PBS to each well.
2. Aspirate and discard the PBS from each well.

Prepare cell lysates

1 Prepare the Cells-to-CT lysate

a. Add 50 µL of Lysis Solution to each sample, then mix the lysis reaction by pipetting up and down 5 times or by gentle shaking on an orbital shaker.

b. Incubate the lysis reaction for 5 minutes at room temperature.

c. Add 5 µL, or 6 µL if using Xeno™ RNA Control, of Stop Solution to each lysis reaction.

1 Prepare the Cells-to-CT lysate *(continued)*

d. Mix the lysis reaction by pipetting up and down five times or by gentle shaking on an orbital shaker.

IMPORTANT! Thoroughly mix the Stop Solution into the lysate.

e. Incubate for 2 minutes at room temperature.

Perform reverse transcription (RT)

1 Perform reverse transcription (RT)

a. In a nuclease-free microcentrifuge tube on ice, prepare an RT Master Mix for the number of reactions required plus 10% overage, according to the following table.

Up to 45% of the RT reaction volume (22.5 µL) can be Cells-to-C_T™ lysate. Adjust the volume of Nuclease-free Water accordingly.

Component	1 reaction	96 reactions[1]	384 reactions[1]
2X Fast Advanced RT Buffer	25 µL	2.64 mL	10.56 mL
20X Fast Advanced RT Enzyme Mix[2]	2.5 µL	264 µL	1.056 mL
Nuclease-free Water	12.5 µL	1.32 mL	5.28 mL
Total	**40 µL**	**4.22 mL**	**16.9 mL**

[1] Volumes include 10% overage.
[2] For the minus-RT control, use Nuclease-free water instead of 20X Fast Advanced RT Enzyme Mix.

b. Distribute RT Master Mix to nuclease-free PCR tubes or wells of a multiwell plate.

c. Add sample lysate to each aliquot of RT Master Mix for a final 50-µL reaction volume.

d. Mix reactions gently, then centrifuge briefly to collect the contents at the bottom of the reaction container.

e. Set up the thermal cycler (or real-time PCR instrument) as indicated in the following table, then load and run the reactions.

Step	Stage	Cycles	Temperature	Time
Reverse transcription (hold)	1	1	37°C	30 minutes
RT inactivation (hold)	2	1	95°C	5 minutes
Hold	3	1	4°C	Indefinite

Perform qPCR

1 Perform qPCR

a. In a nuclease-free microcentrifuge tube at room temperature, prepare the PCR Cocktail plus 10% overage according to the following table.

Component	10 µL PCR reaction	20 µL PCR reaction
PowerUp™ SYBR™ Green Master Mix	5 µL	10 µL
PCR primers Forward and Reverse primers[1]	Variable	Variable
Nuclease-free Water	Variable	Variable
Total	**8 µL**	**16 µL**

[1] Recommended final concentration of each primer is 200–400 nM.

b. Distribute the PCR Cocktail into individual PCR tubes or wells of a real-time PCR plate at room temperature.

c. Add cDNA to the PCR cocktail.

1 Perform qPCR *(continued)*

d. Set up the real-time PCR instrument as indicated in the following table, then load and run the reactions.

Specify SYBR Green fluorescent dye for the experiment.

Step	Stage	Cycles	Temperature	Time
UDG activation	1	1	50°C	2 minutes
Enzyme activation (hold)	2	1	95°C	10 minutes
PCR	3	40	95°C	3 seconds
			60°C	30 seconds
Dissociation curve	4	Use default setting		

IMPORTANT! PowerUp™ SYBR™ Green Master Mix contains ROX™ passive reference dye.

Manufacturer: Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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Revision	Date	Description
A.0	14 June 2018	New document.