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Cells-to-C_T™ 1-Step TaqMan® Kit

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Pub. No. MAN0010751 **Rev.** A.0

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *Cells-to-C_T™ 1-Step TaqMan® Kit User Guide* (Pub. no. MAN0010650). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended for experienced users of the Cells-to-C_T™ 1-Step TaqMan® Kit. For detailed instructions, important procedural guidelines, supplemental procedures, and troubleshooting, refer to the *Cells-to-C_T™ 1-Step TaqMan® Kit User Guide* (Pub. no. MAN0010650).

Before each use of the kit

- Chill 1X PBS on ice, sufficient for 50 µL per 10^5 cells.
- Thaw Stop Solution and bring it to room temperature, with gentle mixing (*do not vortex*).
- (Optional) Just before use, prepare DNase/Lysis Solution master mix (per reaction: 49.5 µL of room temperature Lysis Solution + 0.5 µL of DNase).

Cells-to-C_T™ procedure

1 Prepare cells for lysis

Prepare adherent or suspension cells for lysis.

Cell type	To prepare cells for lysis
Adherent cells grown in 96- or 384-well plates	Aspirate the culture medium, rinse with 50 µL of cold 1X PBS, then aspirate PBS without disturbing the cells.
Cells grown in other vessels, including adherent and suspension cells	1. Detach adherent cells from the culture vessel. 2. Count the cells, pellet, then resuspend the pellet in ~50 µL of chilled 1X PBS per 10^5 cells. 3. Pellet the cells, aspirate the PBS, then resuspend in 5 µL of cold 1X PBS per 10 to 10^5 cells. 4. Distribute 5 µL of cells to a 96-well PCR plate.

2 Prepare the Cells-to-C_T™ lysate

a. Add 50 µL of room-temperature Lysis Solution or DNase/Lysis Solution to the prepared cells, and pipette up and down 5 times to mix well.

b. Incubate at room temperature for 5 minutes.

c. Add 5 µL of room-temperature Stop Solution and pipette up and down 5 times.

d. Incubate at room temperature for 2 minutes.

e. Place the lysates on ice, and proceed to RT-PCR.

STOPPING POINT Lysates can be stored on ice for up to 2 hours or at or below -20°C for up to 5 months.

3 Perform 1-step RT-PCR

a. Thaw all reagents, including previously frozen Cells-to-C_T™ lysates, on ice.

b. On ice, prepare RT-PCR Master Mix for the number of reactions required plus 10% overage.

3 Perform 1-step RT-PCR *(continued)*

Table 1 RT-PCR Master Mix (for 20-µL reactions)

Component	Volume per 20-µL reaction
TaqMan® 1-Step qRT-PCR Mix	5 µL
TaqMan® Gene Expression Assay, 20X	1 µL
Nuclease-Free Water	To 19 µL (for 1 µL of lysate) To 18 µL (for 2 µL of lysate)

c. On ice, add the appropriate volume (18–19 µL) of RT-PCR Master Mix to each sample or control well of an optical reaction plate.

d. Add the appropriate volume (1–2 µL) of lysate or Nuclease-Free Water (for the NTC) to each well (20 µL total).

e. Seal the plate with an optical adhesive cover, vortex the plate for 5–10 seconds, then briefly centrifuge the plate.

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

Table 2 Standard cycling conditions

Step	No. of cycles	Temp.	Time
Reverse transcription	1	50°C	5 min
RT inactivation/initial denaturation	1	95°C	20 sec
Amplification	40	95°C	15 sec
		60°C	1 min

Table 3 Fast cycling conditions

Step	No. of cycles	Temp.	Time
Reverse transcription	1	50°C	5 min
RT inactivation/initial denaturation	1	95°C	20 sec
Amplification	40	95°C	3 sec
		60°C	30 sec