

Cells-to-C_T[™] 1-Step TaqMan[®] Kit

Catalog Number A25605, A25603, A25602

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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 10⁵ 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 | <ol style="list-style-type: none"> 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 10⁵ cells. 3. Pellet the cells, aspirate the PBS, then resuspend in 5 μL of cold 1X PBS per 10 to 10⁵ 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- μ L reactions)

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

- On ice, add the appropriate volume (18–19 μ L) of RT-PCR Master Mix to each sample or control well of an optical reaction plate.
- Add the appropriate volume (1–2 μ L) of lysate or Nuclease-Free Water (for the NTC) to each well (20 μ L total).
- Seal the plate with an optical adhesive cover, vortex the plate for 5–10 seconds, then briefly centrifuge the plate.
- 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 |

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