**Nucleofection of Intestinal Organoids**

1. For testing transduction efficiency, dissociate organoids into single cells using pre-warmed TrypLE™ Express in a 37°C water bath or heat block for 20 minutes. Remove from incubation and pipette mixture up and down 15 - 20 times every 5 minutes to break apart Matrigel® dome and dissociate organoids into a single-cell suspension.
2. Centrifuge cell suspension at 300 x g for 5 minutes.
3. Remove supernatant and resuspend cell pellet in 1 mL DMEM complete media. Run suspension through 70um cell strainer.
4. Count cells. Prepare 1 x 106 cells per electroporation reaction and centrifuge at 300 x g for 5 minutes.
5. Aspirate supernatant from the cell pellet. Resuspend cells in Nucleofection master mix **(Table 1)** and transferred to a 100 µl nucleofection cuvette and pipette up and down gently to mix, trying not to form air bubbles.

**Note:** If air bubbles are present in the cuvette when the cells are electroporated, cell viability and transfection efficiency will be significantly reduced.

**Table 1:** **Nucleofection P3 master mix**

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| --- | --- |
| P3 Primary Cell Nucleofector™ Solution | 82 ul |
| Supplement 1 | 18 ul |
| pmaxGFP | 1 ul |
| Cells | 1\*106 |

1. Set the Lonza® 4D-Nucleofector™ X Unit to program code **DS-138**.
2. Place the Nucleocuvette™ Strip in the Shuttle device of the 4D-Nucleofector™ X Unit, select OK to load the strip, and select Start to begin electroporation.
3. Immediately after electroporation, transfer cells to a DNase- and RNase-free microcentrifuge tube. Add WRN supplemented with 10 µM Y-27632 (ROCK inhibitor, ROCKi, 688000, Merck) to dilute the P3 buffer. Centrifuge at 300 x g for 5 minutes.
4. Seed cell mixture in Matrigel in 2 wells of a 24-well plate and culture with WRN medium with ROCKi (10 µM) for 72 hrs. before FACS analysis.