F-PDO user's manual

F-PDO is cryopreserved in the vapor phase of liquid nitrogen. After receiving an order, the frozen F-PDO is packed on dry ice for transport and delivery.
Start the culture immediately after receipt. If immediate incubation is not possible, F-PDO should be stored in the liquid nitrogen vapor phase. Storage in a -80°C freezer is not recommended.

Note

- For quality, please check the product sheet for each F-PDO.
- Be sure to use the medium indicated on the product sheet.
- Culture the F-PDO according to the methods outlined below.
- We do not guarantee the characteristics and growth of F-PDO if cultured by any method other than the recommended protocol.
- The presence of unknown pathogens is undeniable because of its human origin. A class II safety cabinet for biohazardous materials should be used when cultivating F-PDO. Gloves and lab coats should be worn to prevent infection

Recommended flasks and medium volume

Culture flask		Medium volume
T25 flask	Corning® Ultra-Low attachment cell culture flasks, Cat. 4616	5 mL
T75 flask	Corning® Ultra-Low attachment cell culture flasks, Cat. 3814	15 mL

Thawing and seeding of F-PDO

1. Add 15 mL of medium into a 50-mL sterile tube.
2. Loosen the cap and vent liquid nitrogen from the frozen vial in a biological safety cabinet.
3. Close the cap tightly and wrap the vial using parafilm.
4. Thaw the cells by gentle agitation in a 37°C water bath for 1 minute.
5. Remove the vial from the water bath, wipe it with 70% ethanol, and bring it into the biological safety cabinet.

6. Transfer the vial contents to the sterile centrifuge tube containing 15 mL of medium (mentioned as step 1).
7. Mix the cells and medium gently.
8. Centrifuge at 200 × *g* for 3 minutes at room temperature.
9. Discard the supernatant.
10. Resuspend the cell pellet* in 5 mL of fresh medium and transfer to a T25 flask.
11. Culture in an incubator at 37°C with 5% CO_2.

* Do not pipette vigorously because the pellets disperse with light pipetting.

Depending on the type of F-PDO, it may also be cultured in 6-well plates (2 mL of medium) according to the product sheet.

Medium change

- Change the medium twice a week for a half to full volume. Slow-proliferating cells need only one medium change per week.
- Centrifuge the suspension to precipitate the cells and replace the medium.
- If the cell density is near the upper limit or if the culture medium turns yellow, increase the frequency of medium replacement. If cell density exceeds the upper limit, passage the cells.

For a T25 flask

1. After observing the cells, transfer the flask into a biological safety cabinet.
2. Collect the cell suspension and transfer it into a centrifuge tube.
3. Centrifuge at 200 × *g* for 2 minutes at room temperature.
4. Estimate the volume of the cell pellet. Subculture if the cell density is more than the recommended upper limit indicated on the product sheet. Proceed to "subculture."
    - After thawing F-PDO, the cell density is sometimes above the recommended upper limit; however, allow the cells to grow before the subculture.
5. Discard the appropriate amount of supernatant as indicated on the product sheet.
6. Add fresh medium to a final volume of 5 mL, and suspend the cell pellet.
7. Transfer the cell suspension into a culture flask.
8. Culture the cells at 37°C, 5% CO_2.

Subculture

- Do not use trypsin EDTA or any other dispersing enzymes for passaging.
- If cell density exceeds the upper limit, maintain it by passaging.
- We recommend passaging to a 1:1 dilution of cell suspension in fresh media.
- As it is difficult to measure the number of cells, determine the appropriate cell density based on the size of the cell pellet after centrifugation and determine the timing of passage. The instructions on the product sheet for each F-PDO should be followed.

From one T25 flask to two T25 flasks

1. Work up to step 5 of medium change.
2. Resuspend the cell pellet in 5 mL of fresh medium.
3. Transfer half of the cell suspension volume (2.5 mL) into two flasks.
4. Add 2.5 mL of fresh medium to each flask.
5. Culture at 37°C with 5% CO_2.

The day after medium change, from one T25 flask to two T25 flasks

1. Work up to step 2 of medium change.
2. Transfer the half of the cell suspension volume (2.5 mL) into two flasks.
3. Add 2.5 mL of fresh medium to each flask.
4. Culture at 37°C with 5% CO_2.

From two T25 flask to one T75 flask

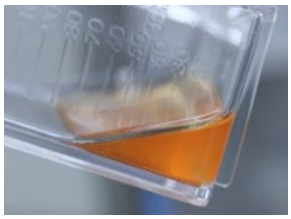
6. Work up to step 5 of medium change.
7. Resuspend the cell pellet in 5 mL of fresh medium.
8. Transfer the cell suspension to a T75 flask.
9. Add 5 mL of fresh medium to the flask.
10. Culture at 37°C with 5% CO_2.

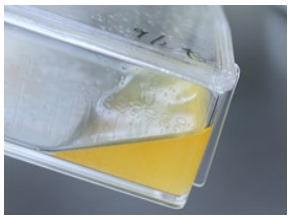
Cryopreservation

1. Observe the cells, then transfer the culture flask into a biological safety cabinet.
2. Transfer the cell suspension into a centrifuge tube.
3. Centrifuge at 200 × *g* for 2 minutes at room temperature.

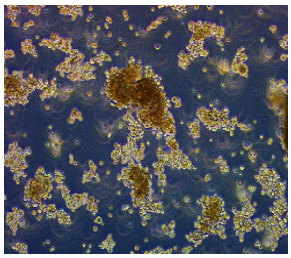
4. Estimate the cell volume from the cell pellet.
   - About 50 µL of the cell pellet in 1 mL of cryopreservation medium is recommended.
5. Discard the supernatant.
6. Add an appropriate volume of cryopreservation medium (CELLBANKER® 2, ZENOAQ RESOURCE), and then suspend the cell pellet.
7. Dispense the cell suspension into a cryotube in each 1 mL.
8. Freeze the cryotube to -80°C at a rate of approximately 1°C per minute using a program freezer or a cryopreservation tube container.
9. Store in the vapor phase of liquid nitrogen after freezing.

Color change in the medium

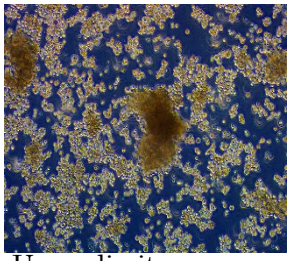




Cell density

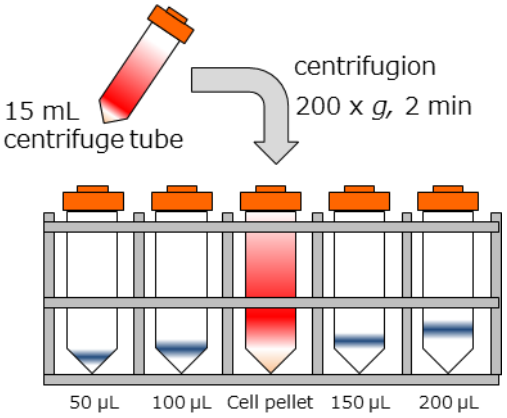


Immediately after passage



Upper limit

Measurement the amount of cells from the pellet size



Line a centrifuge tube containing the cells with tubes marked with the height of 50–200 µL volumes. The amount of cells is determined visually.