Culturing technique for Nunc TripleFlask Treated Cell Culture Flasks

Thermo Scientific™ Nunc™ TripleFlask™ Treated Cell Culture Flasks support conventional flat monolayer culture on three horizontal growth surfaces to maximize growth area. To ensure an equal distribution of cells and medium in each growth layer, prepare a homogeneous cell suspension at the final intended dilution prior to adding cells to the flask. Both cell seeding and harvesting methods are addressed in this technical note.

Description of steps (Figure 1):

1. Prepare a homogeneous cell suspension. Pour the cell suspension into the TripleFlask culture flask against the top surface, tilting the flask slightly to avoid formation of bubbles. The recommended working volume is 100–200 mL.
2. Place the flask in the upright position to allow even distribution of liquid into each compartment.
3. To ensure equal distribution of the cell suspension between the layers, hold the TripleFlask culture flask on its side at an angle (~75°) to the work surface for a few seconds.
4. Quickly but gently turn the flask from its side to the incubation position.
5. During incubation, the culture medium stays equally distributed among the layers.
6. To harvest cells, use a standard trypsinization procedure as in a single-layer flask. Pour 10–15 mL trypsin into the TripleFlask culture flask and distribute as previously described. Once trypsinization is complete, pour the suspension into a new container to recover the cells.

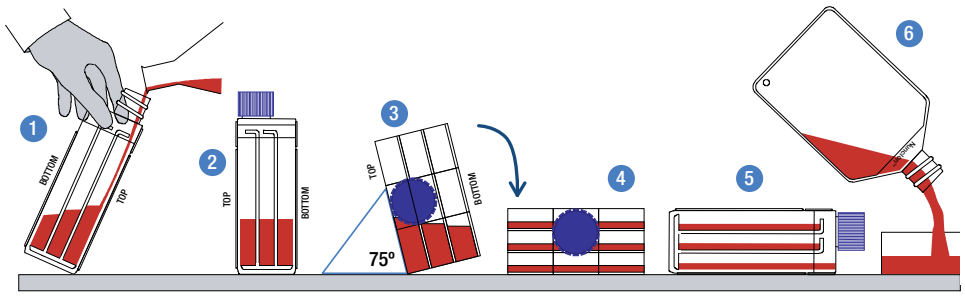

Figure 1. Seeding and harvesting of cells cultured in a TripleFlask culture flask.

Detailed protocols

To seed cells

1. Prepare a homogeneous cell suspension in a convenient vessel for dispensing. Gently swirl, avoiding bubble formation.
2. Tilt the flask slightly (less than 45°).
3. Pour the cell suspension slowly and steadily into the flask against the top surface. Avoid rim contact, and avoid bubble formation.
4. Remount the cap without touching the neck of the flask.
5. Stand the flask upright on end to allow the liquid volume to be distributed between compartments.
6. Once the suspension is distributed, rest the flask on its side, and then hold the flask at a 75° angle to the work surface for a few seconds to help ensure equal, final distribution (see Figure 1, step 3).
7. Quickly but gently tilt the flask to the growth position (with the Nunclon™ imprint facing up) to distribute the cell suspension equally to each level.
8. Incubate as usual.

To harvest cells

1. Stand the flask upright on end.
2. Pour the medium into a waste receptacle.
3. Rinse the monolayers with PBS or other buffer: pour the buffer into the flask, rock the flask gently to rinse all three layers, then drain.
4. Add 10–15 mL trypsin or other dissociation reagent to the flask. Rock the flask to distribute the trypsin evenly across each layer. Then pour off the excess trypsin.
5. Incubate at 37°C for 1–2 min or as usual.
6. (Optional) Dislodge the cells by tapping the flask with the palm of your hand.
7. Rinse the cells from the flask with an adequate volume of fresh medium containing serum, rocking to dislodge the cells.
8. Once resuspended, pour the harvested cells into a sterile container.

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Ordering information

Code	Description	Pack/Case	Offer Price, £
132867	TripleFlask Treated Cell Culture Flask, solid cap - Culture area 500 cm^2, working volume 200 mL	2/32	240.00
132913	TripleFlask Treated Cell Culture Flask, filter cap - Culture area 500 cm^2, working volume 200 mL	2/32	240.00
132920	TripleFlask Treated Cell Culture Flask, filter cap, barcoded - Culture area 500 cm^2, working volume 200 mL	2/32	286.67
132925	TripleFlask Non-treated Cell Culture Flask, filter cap - Culture area 500 cm^2, working volume 200 mL	2/32	286.67

Offer valid until 30th September 2023.

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