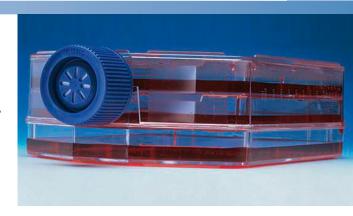
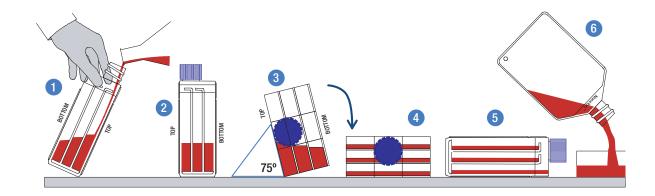
# Thermo Scientific Nunc TripleFlask Culturing Technique

The Thermo Scientific™ Nunc™ TripleFlask™ employs a conventional flat monolayer culture on three horizontal growth surfaces to maximize growth area. To ensure an equal distribution of cells and media 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 Tech Note.



## **Description of steps in diagram:**

- Prepare a homogeneous cell suspension. Pour the cell suspension into the TripleFlask against the top surface, tilting flask slightly to avoid foam or bubbles. Recommended working volume is 100-200 mL.
- 2. Leave the flask in the upright position for a short time to allow equilibration of liquid in each compartment.
- 3. To ensure equal distribution of cell suspension between layers, it is recommended to hold the TripleFlask on its side for a short time, at an approximately 75° angle to the work surface.
- 4. Quickly, but gently, turn the flask from its side to the incubation position.
- 5. During incubation, an equal distribution of culture media is maintained on each layer.
- 6. To harvest cells, use a standard trypsinization procedure as in a single-layer flask. Pour 10-15 mL Trypsin into the TripleFlask and distribute as above. Once trypsinization is complete, pour the resulting suspension into a new container to recover cells.





#### **Details**

#### **To Seed Cells**

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

#### To Harvest

- 1. Stand flask on end.
- 2. Pour medium into waste receptacle.
- 3. Rinse monolayers with PBS or standard buffer.
  - Pour buffer into the flask.
  - Rock flask gently to rinse all three layers, then drain.
- 4. Add 10-15 mL Trypsin or other dissociation reagent to the cell suspension. Rock the flask to distribute Trypsin evenly across each layer. Pour off excess Trypsin.
- 5. Incubate at 37°C for one to two minutes or as usual.
- [Optional] Dislodge cells by tapping flask with palm of hand.
- Rinse cells from flask with an adequate volume of fresh medium containing serum, rocking to dislodge cells.
- 8. Once resuspended, pour cell harvest into a sterile container.

# **Ordering Information**

| Product Name                     | Product Features  | Thermo Scientific<br>Cat. No.<br>Units/pack/case<br>1/32 | Thermo Scientific<br>Cat. No.<br>Units/pack/case<br>4/32 |
|----------------------------------|---|--|--|
| TripleFlask, Vent/Close Cap      | Culture area 500 cm <sup>2</sup> ,<br>Working volume 200 mL | 132867   | 132925   |
| TripleFlask, Filter Cap          | Culture area 500 cm <sup>2</sup> ,<br>Working volume 200 mL | 132913   | 132865   |
| Barcoded TripleFlask, Filter Cap | Culture area 500 cm <sup>2</sup> ,<br>Working volume 200 mL | 132920   | 132935   |

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