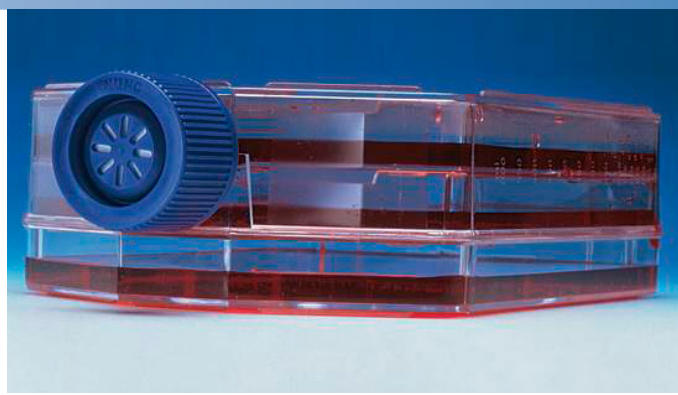
Tech Note

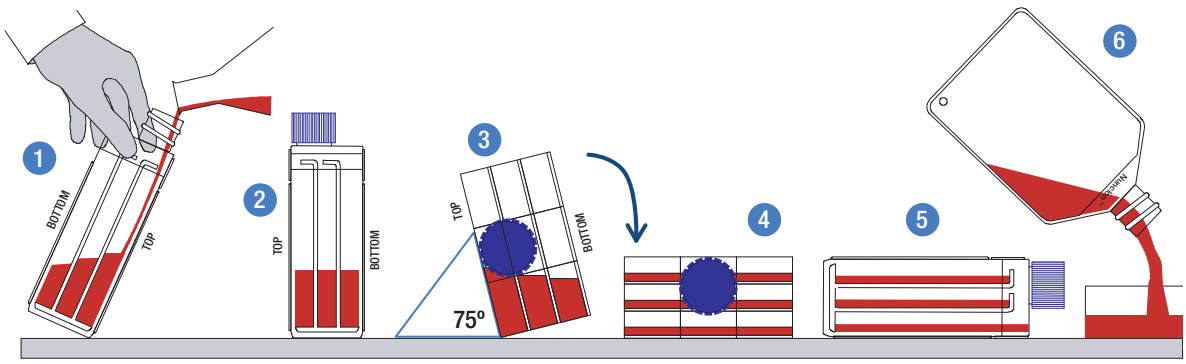
Thermo Scientific Nunc TripleFlask Culturing Technique



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:

1. 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.



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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.
5. 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).
7. 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.
6. [Optional] Dislodge cells by tapping flask with palm of hand.
7. 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 Cat. No. Units/pack/case 1/32	Thermo Scientific Cat. No. Units/pack/case 4/32
TripleFlask, Vent/Close Cap	Culture area 500 cm^2, Working volume 200 mL	132867	132925
TripleFlask, Filter Cap	Culture area 500 cm^2, Working volume 200 mL	132913	132865
Barcoded TripleFlask, Filter Cap	Culture area 500 cm^2, Working volume 200 mL	132920	132935

thermoscientific.com/tripleflask

ANZ: Australia: 1300 735 292, New Zealand: 0800 933 966; **Asia:** China Toll-free: 800-810-5118 or 400-650-5118; India: +91 22 6716 2200, India Toll-free: 1 800 22 8374; Japan: +81-3-5826-1616; Other Asian countries: 65 68729717
Europe: Austria: +43 1 801 40 0; Belgium: +32 2 482 30 30; Denmark: +45 4631 2000; France: +33 2 2803 2180; Germany: +49 6184 90 6000, Germany Toll-free: 0800 1-536 376; Italy: +39 02 95059 554; Netherlands: +31 76 571 4440; Nordic/Baltic countries: +358 9 329 10200; Russia/CIS: +7 (812) 703 42 15; Spain/Portugal: +34 93 223 09 18; Switzerland: +41 44 454 12 22; UK/Ireland: +44 870 609 9203
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