Tech Note
No. 02

Thermo Scientific Nunc Nunclon Δ TripleFlask Culturing Technique

The Thermo Scientific Nunc Nunclon Δ TripleFlask employs conventional flat monolayer culturing on three horizontal growth surfaces. To ensure equal distribution of cells and media in each growth chamber, prepare a homogeneous cell suspension, add to flask.

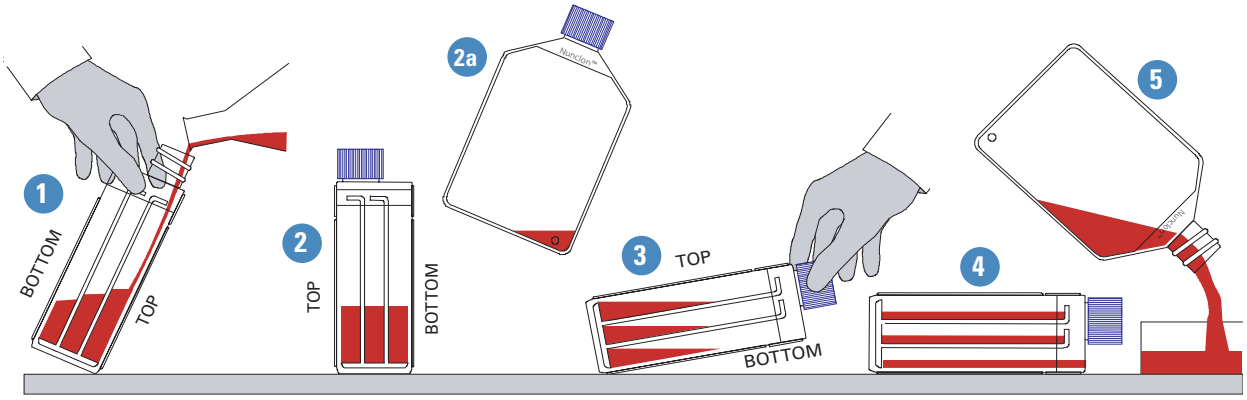
Both inoculating and cell harvesting methods are addressed in this Tech Note.

Materials

Nunc™ Nunclon™ Δ TripleFlask (Cat. Nos. 132867 and 132913)

Medium*:	0.2-0.4 mL/cm² culture area or 100-200 mL per TripleFlask total
Cells*:	Resuspended in complete medium
	Seed at usual density (cells/cm²)
Trypsin*:	0.1% to 0.25% (± 10 mm EDTA)
EDTA*:	0.1-2.0% in HBSS or PBS without Ca^{++} or Mg^{++}

** medium, reagents and cells as determined by previous standard culture conditions*



1. Prepare homogeneous cell suspension. Pour into the TripleFlask, 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

2a. The flask may be canted momentarily around the connecting channel corner for facilitating the equilibration of small volumes

3. Quickly, but gently place the flask in the incubation position
4. The liquid is equally distributed over the three growth surfaces
5. The flask is emptied in the same way as a conventional flask. To harvest cells add 10-15 mL Trypsin

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Method

To Inoculate

1. Line up TripleFlask, standing on end with the same orientation for each flask bottom (i.e. all bottoms facing left).
   - Loosen caps, but do not remove completely.
   - Position line of flasks, leaving sufficient front work space clear. Avoid passing hands and utensils over flasks.
   - Keep caps on flasks whenever possible.

2. Prepare homogeneous cell suspension in a convenient vessel for dispensing.
   - Gently swirl, avoiding bubble or foam formation.

3. Pull first flask to be inoculated out of the line of flasks. Grip cap between little finger and palm.

   Lift off, keeping cap interior pointed downward.

4. Tilt flask slightly (less than 45°), with side or bottom of flask facing palm of hand.

5. Pour cell suspension slowly and steadily into flask.
   - Pour cell suspension into body of flask, avoiding rim contact.
   - Medium should flow down top side of flask (surface with Nunclon imprint).
   - **Avoid bubble formation.**

6. Remount cap without touching the neck of the flask.

7. Stand flask on end to allow medium equilibration between compartments.

8. Quickly, but gently tilt flask into growth position (Nunclon imprint facing up) to distribute cells and medium equally onto each level.

9. Incubate as usual.

To Harvest

1. Stand flask on end. Grip flask with palm facing side or bottom of flask.

2. Pour medium into receptacle. Medium should flow against flask top.

3. Rinse monolayers with PBS or standard buffer.
   - Add rinse as you would cell inoculum.
   - Rock flask gently.
   - Drain as above.

4. Add 10-20 mL Trypsin or other reagent as usual. Rock to distribute evenly. Pour off excess.

5. Incubate at 37°C for 1-2 minutes or as usual.

6. Dislodge cells by tapping flask with palm of hand.

7. Rinse cells from flask, rocking to dislodge cells and pouring to collect harvest medium.

8. For other harvesting options, see Thermo Scientific Nunc Tech Note No. 3: *Non-enzymatic Methods for Cell Harvesting.*

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