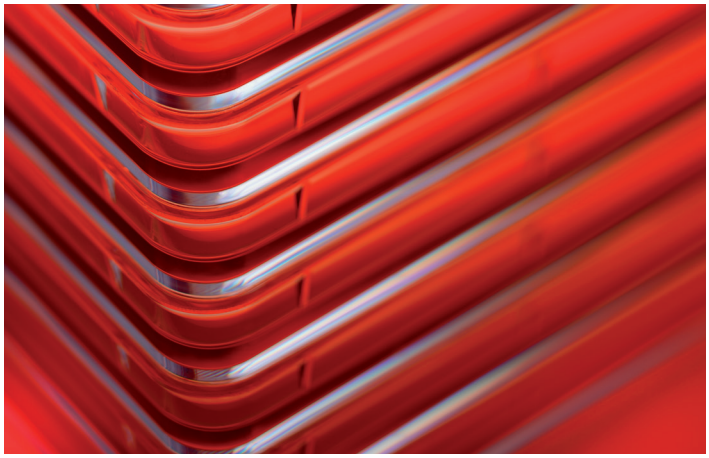
Harvesting adherence-dependent cells from Nunc Cell Factory systems

Introduction

The Thermo Scientific™ Nunc™ Cell Factory™ system is a large multilayer unit designed for culturing adherent cells. The design of a Nunc Cell Factory system is similar to a cell culture flask; therefore, a standard cell-harvesting protocol may be used. With minor modifications, a standard protocol can be optimized for the unique features of the Nunc Cell Factory system.



Incubation, monitoring, and use of a parallel culture

Establishing a parallel culture in either a Nunc Cell Factory 1-layer system or a Thermo Scientific™ Nunc™ flask is recommended as a positive control for cell viability and to assist with determining the proper incubation time for your primary culture. The parallel culture should be maintained under identical conditions, including cell line, cell source, seeding density, medium type, medium volume (mL/cm^2), incubation temperature, and time. During harvest, identical protocols should be applied to both the primary culture and the parallel culture. Monitor cell dissociation by placing the parallel culture vessel over the stage of an inverted microscope and record the total incubation time.

Note: To mimic the primary culture within the Cell Factory system, do not add solutions to the cell sheet. Ensure the Cell Factory system remains in a parallel position while on the microscope stage to prevent cell detachment resulting from physical forces. Turn on the light of the microscope when monitoring cells, and turn off the light immediately when finished, to prevent excessive heating of the vessel. The incubation time determined from the parallel culture may be used to establish the optimal time for harvesting your primary culture.

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Material preparation

1. **Prewarm rinsing and dissociation solutions to room temperature:** To obtain uniform cell dissociation from all layers, the rinsing and dissociation solutions should be prewarmed to room temperature.

2. **Remove and discard culture medium.**

3. **Rinse:** Rinse cell sheets with a balanced salt solution to remove remaining medium and serum from the cell culture surface.

Recommend volume*	Example of solution
30–40 mL/layer	Calcium- and magnesium-free balanced salt solution

4. **Dissociate cells:** To initiate cell detachment, add your preferred cell dissociation solution to the Cell Factory system.

Recommend volume*	Example of solution
20 mL/layer	Trypsin, EDTA

5. **Stop dissociation:** Gently rock the Cell Factory system back and forth to release cells from the surface. When the cells have detached, add a stopping solutions and continue to gently agitate the Cell Factory system to complete detachment and prevent cell clumps.

Note: When rocking the system, take care to prevent the cell suspension from coming into contact with the top surface of each layer. It is difficult to recover cells from the top surface.

Recommend volume*	Example of solution
25 mL/layer	Cell culture medium

6. **Harvest cell suspension from the Cell Factory system.**

* Solution volumes: To sufficiently cover the entire surface of all layers, we recommend the indicated volumes.

Find out more at **thermofisher.com/cellfactory**

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