

# Harvesting adherent-dependent cells from Thermo Scientific Nunc Cell Factory Systems

## Introduction

The Thermo Scientific™ Nunc™ Cell Factory™ system is a large multi-layer unit designed for the culture of 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 the use of a parallel culture:

Establishing a parallel culture in either a Nunc Cell Factory 1 or 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<sup>2</sup>), incubation temperature and time.

During harvest, identical protocols should also be applied to the parallel culture. Monitor cell dissociation by placing the parallel culture vessel over the stage of reversed microscope and record the total incubation time.

*Please note: To mimic the primary culture within the Cell Factory system, do not add solutions to the cell sheet. Ensure that 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 microscope when monitoring cells and turn off light immediately when finished, to prevent heat up of the vessel. The incubation time determined from the parallel culture may be used in establishing the optimal time for harvesting your primary culture.*



### 1. Material preparation:

Pre-warm rinsing and dissociation solutions to room temperature:

To obtain uniform cell dissociation from all layers, it is recommended that the rinsing and dissociation solutions be pre-warmed to room temperature.

### 2. Remove and discard culture medium.

### 3. Rinsing:

Rinse cell sheets with a pre-balance rinsing solution to remove remaining medium and serum from the cell culture surface

Volume Recommendation*	Solution Examples
30-40mL/layer	Calcium- and magnesium-free balanced salt solution

### 4. Cell dissociation:

To initiate cell detachment, add your preferred cell dissociation solution to the Cell Factory system.

Volume Recommendation*	Solution Examples
20mL/layer	Trypsin, EDTA

### 5. Stopping dissociation:

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

*Please 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 top surface.*

Volume Recommendation*	Solution Examples
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 above volumes

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