

# An examination of Nunc Cell Factory systems for consistent, reliable, production of large quantities of cells

Key words: Cell culture, bioproduction, cell density, MDCK, MDBK, VERO, CHO, multilayer, Cell Factory system

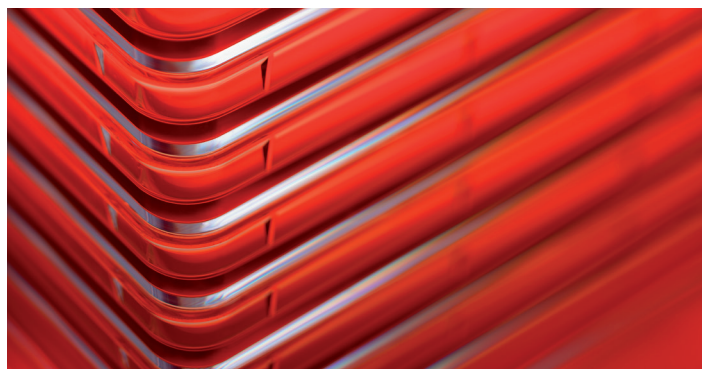
## Introduction

Thermo Scientific™ Nunc™ Cell Factory™ systems are culturing solutions designed for adherent cell cultures. These systems consist of multiple polystyrene growth surfaces, assembled together to maximize the surface area available for cell growth, while minimizing the incubator volume required for maintaining cells. Cell Factory systems are popular in fields where large quantities of cells are required and are a reliable tool for the production of vaccines, and recombinant proteins, and for the generation of cell mass.

Unlike single-layer systems, where the entire culture is easily visualized under a microscope, the middle layers of a multilayer system are obscured from visualization by the surrounding layers. As a result of this design, it can be difficult to monitor the culture conditions and cell growth on these layers, or verify that cultures grow consistently between layers. Here, we examine the growth of several commonly used cell lines within 10-layer Cell Factory systems by physically separating and photographing each layer after a typical period of cell growth. This protocol enabled the verification of consistent cell growth throughout all Cell Factory system layers.

## Materials

Materials	Cat. No.
10-layer Cell Factory system	170009
Chinese hamster ovary (CHO) cells	ATCC CCL-61
Bovine kidney (MDBK) cells	ATCC CCL-22
Canine kidney (MDCK) cells	ATCC CCL-34
Monkey kidney (VERO) cells	ATCC CCL-81
MEM/EBSS	SH30024.02
Non-essential amino acids (NEAA)	M7145
Sodium pyruvate	SH30239.01
Antibiotic antimycotic solution (AAS)	5955
Fetal bovine serum (FBS)	SH30910.03 SH30396.03



Cells were harvested from a source culture using a standard trypsin harvesting procedure, and cell density was determined using an automated cell counter. Cells were then diluted in prepared media to 30,000 cells/mL for CHO, MDBK, and VERO cells, or 45,000 cells/mL for MDCK cells. 2,000 mL of diluted cell suspension was seeded into each Cell Factory system. All Cell Factory systems were closed and incubated in a warm room at 37°C for 4 days. The warm room did not contain a CO<sub>2</sub>-controlled environment.

Once incubation was complete, all media was removed from the Cell Factory system, and cells were stained using crystal violet. After staining, the layers were separated using a hot knife and each individual layer was photographed to examine cell growth on the entire layer (Figure 1). Photomicrographs were also taken of the stained cells at 3 positions on each layer (data not shown).

Observation of the stain density over the entire surface of each layer of the Cell Factory system shows very consistent growth between all layers for all cell types (Figure 1). Layers 1 and 5 show similar growth characteristics in all cell types. The cell density was similar in all layers except on layer 10 for all cell types (images not shown). While the bottom layers of the multilayer culture vessels show cell densities that would produce cell yields similar to all other layers, in most cases, a pattern emerges of variable cell density that resembles a wave. This pattern is typically seen when culturing cells, and is found specifically within this layer due to its contact with the incubator shelf. The vibrations from the incubator, as well as exterior sources, transmit waveforms into the liquid growth media. While these are not necessarily detrimental to cell growth, they can be minimized by reducing vibration as much as possible from the culture conditions.

## Results and discussion

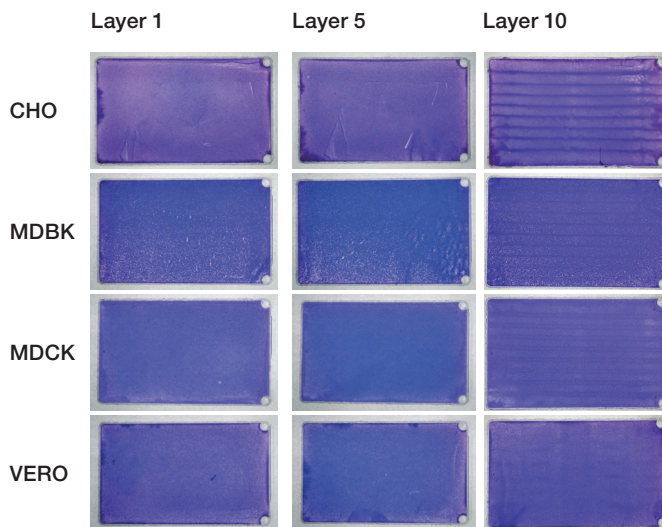
Overall cell density in each layer was observed qualitatively through staining the layers of cells, making any deficiencies in the cell growth visible. Photomicrographs of the cell types tested show similar cell density and morphology throughout all layers of the 10-layer Cell Factory systems. Figure 2 shows images from the top (layer 1), middle (layer 5), and bottom (layer 10) of each cell type. All other layers showed similar results (images not shown).

## Conclusion

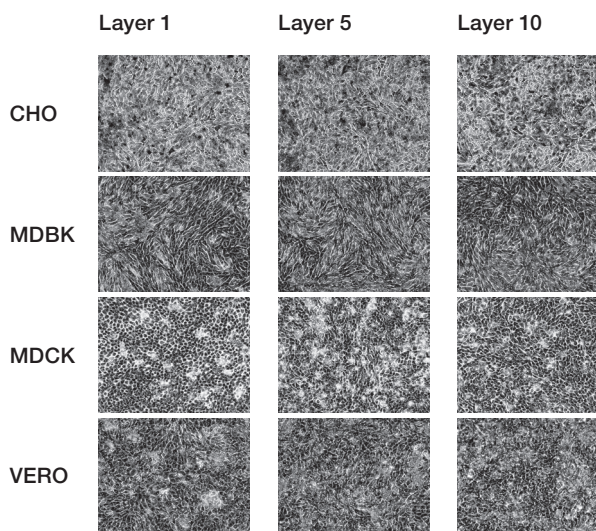
The photographs and photomicrograph images of the individual layers of a 10-layer Cell Factory system provide visual confirmation that the cell culture conditions are consistent from layer to layer and that there are no significant differences in:

- Cell attachment
- Growth
- Density
- Morphology

The multilayered Nunc Cell Factory system is an ideal solution for consistent reliable production of large quantities of cells.



**Figure 1. Photographs showing cell density of the entire growth surface of the top, middle, and bottom layers of the Cell Factory.** The growth pattern on the bottom layer is due to vibrational effects during incubation; however, this is not detrimental to cell growth.



**Figure 2. Photomicrographs showing similar cell density and morphology in the top, middle, and bottom layers of the Cell Factory.**

Find out more at [thermofisher.com/cellfactory](https://thermofisher.com/cellfactory)

**ThermoFisher**  
SCIENTIFIC