# Harvesting Cells from Corning® CellSTACK® Culture Chambers

Protocol

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## Introduction

Harvesting cells from multilayer CellSTACK culture chambers that do not allow direct pipette access to the cells can be challenging. The inability to view the cells microscopically when working with the larger size (10 and 40 layer) vessel adds to the challenge. This protocol covers some basic techniques and suggestions for harvesting cells from CellSTACK culture chambers. For optimal cell yields and viability, it is important to optimize the harvesting protocol for each cell line.

A phosphate-buffered saline is used for rinsing since it maintains a physiological pH without requiring a closed system (required by buffers based on Hanks' saline) or gassing with carbon dioxide (required by buffers based on Earle's saline). Calcium and magnesium are omitted because these play an important role in cell attachment.

## Sterile Solutions

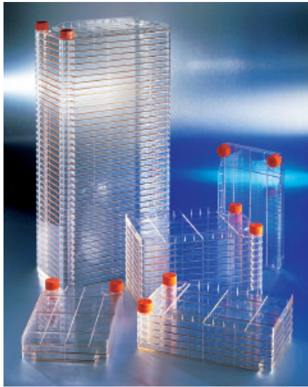
1. *Cell culture medium.* This should contain all of the additives (fetal bovine serum, glutamine, etc.) required by the cells.
2. *Calcium- and Magnesium-Free Phosphate-Buffered Saline CMF-PBS.* This simple salt solution is used to maintain proper pH and osmotic balance while the cells are being washed to remove protease inhibitors that are found in most animal sera. We recommend using 15 to 30 mL/layer (0.024 to 0.047 mL/cm$^2$).
3. *Dissociation solution.* As a starting point, use the same dissociating solution and concentration that is used for harvesting these cells from other vessels. The use of chelating agents, such as EDTA, in addition to the dissociating enzymes normally used, may increase the effectiveness of the harvesting procedure. We recommend using 15 to 30 mL/layer (0.024 to 0.047 mL/cm$^2$).
4. *Appropriate inhibitors for dissociating agents.* These are especially important if the cells are not washed after harvesting. We recommend using a volume equal to the dissociation solution to dilute/ neutralize the dissociation reagent.

The recommended amounts of CMF-PBS and dissociating solution are starting volumes only. If cells are relatively easy to harvest from the chambers, then the amounts used for future harvests may be reduced by up to 50% or more.

## Cell Harvesting Procedure

The goal is to remove the cells from the plastic substrate and break cell-to-cell bonds as gently as possible. There are several variations of this protocol dependent on the methods (pouring, pumping, etc.) you prefer for removing and adding solutions to the Corning CellSTACK chambers. *This protocol is designed for harvesting cells from a 10-layer CellSTACK chamber; amounts used for other chamber sizes should be changed accordingly*. We recommend growing additional cell cultures under identical conditions (cell density and medium amounts) in a companion vessel (a flask or CellSTACK 1-Stack chamber) as an aid for monitoring both cell growth and harvesting.

1. Remove and discard the culture medium. See the *CellSTACK Instructions for Use* or the Video Guide on the Corning Life Sciences web site for detailed information on general filling and emptying techniques for CellSTACK chambers.



Corning® CellSTACK® culture chambers are available in 5 sizes: 1-, 2-, 5-, 10-, and 40-layer chambers.

Corning 500 mL centrifuge tube (Cat. No. 431123) with optional support cushion

Corning sterile polystyrene storage bottles

Storing cells on ice will slow cell metabolism. This will improve cell viability and reduce cell clumping.

2. Rinse monolayer.
   a. Add the recommended volume of CMF-PBS to the 10-layer CellSTACK chamber. Recap tightly and distribute the CMF-PBS equally to each chamber layer by laying the vessel on its longest side (liquid will come in contact with cap) (see CellSTACK Instructions for Use, Figure 3).
   b. Once the liquid has equilibrated, stand up the vessel (see see CellSTACK Instructions for Use, Figure 4) to separate the layers, then slowly set vessel down to incubation position vessel (CellSTACK instruction for use figure 5). Tilt the chamber back and forth in both directions to thoroughly rinse each layer and remove all traces of the old medium.
   c. Remove and discard the wash solution. A second rinse with CMF-PBS is highly recommended for cells that are difficult to harvest.
3. Dissociate the monolayer.
   a. Add the recommended volume of prewarmed dissociating solution to the chamber and distribute equally to each chamber by following rinsing steps. Prewarming of the dissociating solution will decrease the required exposure period.
   b. Tapping on the chamber's sides may help detach the cells from the surface.
4. Gently tilt the chamber side to side and end to end to distribute the dissociating solution evenly across each layer. Tapping on the chamber's sides may help detach the cells from the surface. Because it is impossible to directly monitor the action of the dissociating solution on the cells in a 10-layer chamber, we recommend growing the same cells under identical conditions (relative cell density and medium amounts) in a companion vessel. This companion vessel (usually a flask or 1-layer CellSTACK chamber) can then be treated in parallel for monitoring both cell growth and progress of the cell harvesting procedure.
5. Collect the dissociating solution containing the resuspended cells in 250 or 500 mL centrifuge tubes (Corning Cat. No. 430776 or 431123) or 1L plastic or glass storage bottles (Corning Cat. No. 430518 or 1395-1L). Neutralize the dissociating agent (if possible) and place the cells on ice. To recover additional detached cells that have been left behind in the chamber, one or more additional rinses are recommended:
   a. For easy to remove cells, this rinse step can be done with CMF-PBS or medium which is then added to the cell suspension from the first dissociation. If a substantial number of viable cells are found in the rinsing solution, then a second rinse should be done or the dissociating solution or harvesting procedure may need to be adjusted. (See step b below.) If the rinsing solution contains few viable cells, then this step can be omitted in the future.
   b. For very difficult to remove cells, rinsing should be done with additional prewarmed dissociating solution. It may be necessary to incubate the cells for a few minutes to give the solution time to act on the remaining attached cells. If a substantial number of viable cells are found in the second harvest, then the original dissociating solution or harvesting procedure may need to be adjusted. If the second harvest contains few viable cells, then this step can be omitted in the future.
6. Inactivate or remove the dissociating agents. Some dissociating agents should be immediately removed by centrifugation to prevent carry over which can cause poor cell attachment or toxicity, especially in combination with serum-free medium. However, other dissociating agents, such as trypsin, can be inactivated by adding chilled medium with serum or a trypsin inhibitor and do not need to be removed. If removal is desired, spin the cell suspension at 100 x g for 5 minutes. Then remove the medium containing the dissociating agent and replace with fresh medium.

7. Count the cells to determine cell yield and viability. If cell yield is lower than expected or cells are still attached in the chamber, you need to:
   a. check if dissociating solution was prewarmed;
   b. increase the concentration, volume or incubation time of the dissociating agent;
   c. add a chelator, such as EDTA, to the dissociating solution;
   d. add an additional washing step with CMF-PBS either prior to adding, or after removing the dissociating solution.

   If cell viability is low, you need to re-examine the harvesting process to determine if:
   a. the dissociating solution is too harsh;
   b. the process for removing the cell suspension is causing damage;
   c. the centrifugation step is too hard or too long;
   d. the dissociating solution is not being removed or inactivated.

## References

Freshney, R. I., (2000). Culture of Animal Cells: A Manual of Basic Technique, (4th edition); Wiley-Liss, New York, pages 413-414.

Please visit the Corning Technical web site (**www.corning.com/lifesciences**) to download these and other useful cell culture articles and protocols:

**Calcium Phosphate Transfections in Corning® CellSTACK® Culture Chambers**
This protocol is a generalized procedure for calcium phosphate based transfection in Corning CellSTACK Chambers. It is intended as a starting point for individuals wishing to transfect large numbers of cells in the Corning CellSTACK chamber and can be adapted for each size.

**Mycoplasma Detection using DNA Staining**
This protocol provides a detailed procedure for using Hoechst stain #33258 to stain the DNA in cell monolayers for detecting mycoplasmas or other prokaryotic organisms.

**Subculturing Monolayer Cell Cultures**
This protocol provides a detailed procedure for subculturing attached cells using enzymatic dissociation. It explains the theory behind some of the keys steps in the process.

**Cryogenic Preservation and Storage of Animal Cells**
This protocol provides a detailed procedure for cryogenically storing a variety of animal cells.

**Single Cell Cloning by Serial Dilution**
This protocol describes a simple approach using a 96 well plate to isolate colonies derived from single cells.

**Clonal Growth of Cells in Semisolid Media**
This protocol provides a detailed procedure for growing animal cells in agar-based media for either transformation assays or cloning.

**Use of Corning Cloning Cylinders for Harvesting Cell Colonies**
This protocol describes how to isolate and develop a population of cells (a clone) that are the descendants of a single cell.

**Single Cell Isolation Using Glass Chips**
This protocol describes a simple approach using glass coverslip chips to isolate colonies derived from single cells.

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