



Product Information

SIGMA-SOLOHILL MICROCARRIER BEADS Pronectin® F Coated Beads

Catalog No. **Z37866-6**
Store at Room Temperature

Product Description

Pronectin®-F coated beads have an extremely thin layer of recombinant, **non-animal source** polymer that incorporates multiple copies of the RGD attachment ligand of human fibronectin interspaced between repeated structural peptide units. This coating promotes cell attachment even for weakly adhering cell lines. The core bead is a solid copolymer sized 125-212 microns in diameter with a specific gravity of 1.02. This design prevents absorption of toxic materials during long-term use and eliminates bead fragmentation during freeze-thaw, ultrasonic, or aggressive mechanical harvesting. Optimized for the stringent requirements of pharmaceutical applications, these beads also perform well under serum-free and protein-free media conditions.

Additional key features of Pronectin® F coated beads include: suitability for bioreactors and smaller stirred vessels; easier proteolytic cell harvesting than from porous beads; solid bead matrix requiring no hydration before use; and autoclavable *in situ* using deionized water or phosphate buffered saline.

REAGENT

For laboratory use only.
Not for drug, household or other use.

Preparation Instructions

The following is a general protocol which is recommended to be used to make a 200 ml suspension culture in a spinner flask. Cell cultures at bead loadings of 5-40 grams per liter have been demonstrated successfully. Twenty (20) grams per liter is suggested as a starting point. Therefore, a 200 ml culture will require 4 grams of beads. Except for weighing and suspending the microcarrier beads, aseptic techniques should be used throughout the protocol. Bead hydration is not required.

1. Clean, siliconize and autoclave all glassware and pipettes. Siliconizing glassware will prevent cell attachment to the treated glassware. Any commercially available siliconizing agent is acceptable.
2. Suspend 4 grams of microcarrier beads in deionized or distilled water or calcium/ magnesium

free phosphate buffered saline solution (CMF-PBS) and autoclave at 121 °C or 131 °C for 15 minutes on the liquid cycle.

3. Rinse the beads in a small amount of media. The intent is to both rinse away the autoclaving liquid and to condition the beads with the media. Acclimate the microcarrier/media solution in the CO₂ incubator (37 °C) for a minimum of 30 minutes. Discard this media and resuspend the beads in fresh, warm media.

Procedure

Cell Attachment

1. The cell inoculum is generally 1×10^5 cells/ml. For a total culture volume of 200 ml, 2×10^7 cells are needed. Add cells to the warm, microcarrier bead/media suspension and add enough warm media to make 100 ml. The cells should be in the log phase for optimum attachment and growth. The attachment phase of the spinner culture should occur at 2 volume to facilitate cell-to-bead interactions. Stir as slowly as possible while preventing the bead/cell slurry from forming a static layer on the bottom of the stir flask. For fastidious cells which attach more slowly, an intermittent stirring protocol may be required. If cells are slow to attach and spread in monolayer, they will be slow to attach on the microcarriers.
2. Stir the incubated spinner flask at 18-21 rpm for a minimum of 6 hours. Frequently, the spinner flask runs overnight (e.g., 12-14 hours). If your spinner system allows intermittent stirring, use it. It is recommended to set the stir cycle at 1 minute on and 20-30 minutes off. Bring the volume to 200 ml with fresh, warm media.

Cell Maintenance

Maintain the cells as required for their growth and metabolism. Generally, one half media exchange is needed every second day.

Cell Harvesting

Sigma-SoloHill microcarriers have a surface from which cells can be as efficiently and gently removed as from other plastic surfaces. The following protocol has been successfully used to harvest a variety of cells from suspension culture flasks (100 - 1000 ml). Cells can be dislodged or harvested from Pronectin7 F coated substrates using standard trypsinization procedures.

1. Allow the microcarriers to settle and quickly decant the medium from the suspension culture flask. Gently rinse the microcarriers in a small volume of calcium/magnesium-free phosphate-buffered saline solution (CMF-PBS) or serum-free media.
2. Resuspend the cells in a solution of a proteolytic enzyme. A 0.25% solution of trypsin in CMF-PBS is most often used. Other enzymes and other concentrations may be used depending on the characteristics of the cells in question. Additionally EDTA is often added to facilitate the removal of divalent cations. The amount of trypsin depends on the quantity of beads in the spinner culture. As a guideline, use equal volumes of enzyme solution and cell/bead slurry. For example, four grams of beads (20 g/liter loading and a 200 ml culture) will occupy about 6 ml. Therefore, about 6 ml of enzyme solution is used.
3. The cells and microcarriers are incubated in the trypsin solution for a brief period of time. For example, for most fibroblastic cells 1-2 minutes may be sufficient although 5-10 minutes may be necessary for epithelial cells. Optimal dissociation time must be determined for each cell line or cell type. The beads may require gentle agitation to release the cells from the microcarrier surface.
4. After the cells are released from the microcarriers, the trypsin solution should be diluted with serum containing culture media to inhibit further trypsin activity. If other enzymes are used instead of trypsin, appropriate measures should be taken to inhibit the enzymes. The cells and microcarriers are then centrifuged and resuspended in culture media.
5. The cells can be separated from the microcarriers by filtration through a suitable nylon, Teflon or stainless steel screen.

This general protocol may be modified to reflect unique characteristics of any given cell. An advantage of Sigma-SoloHill microcarriers is that cells will exhibit the same characteristics on these beads as they do on plastic surfaces. Information gained from studies in conventional monolayer culture will be directly applicable to the use of these microcarrier beads.

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