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ProductInformation

Sigma-Solohill Microcarrier Beads
FACT Coated

Catalog Number **Z378682**
Store at Room Temperature

Product Description

Fast Attachment Collagen-Treated (FACT) microcarrier beads have an extremely thin layer of porcine collagen, which has been surface modified by a proprietary method. This modification greatly enhances cell attachment and cell spreading. Attachment occurs much quicker and spreading is rapid due to the presence of the collagen matrix.

The core bead is a solid copolymer sized 125–212 µm 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.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The following is a general protocol recommended 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 <u>not</u> 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. Discard the autoclaving liquid and rinse the beads in a small amount of medium. The intent is to both rinse away the autoclaving liquid and to condition the beads with the medium. Multiple rinses are commonly used and will eliminate debris or precipitants if present.
4. Acclimate the microcarrier/medium solution in the CO_2 incubator (37 °C) for a minimum of 30 minutes. Discard this medium and resuspend the beads in fresh, warm medium.

Storage/Stability

Store the product at room temperature.

Procedure

<u>Cell Attachment</u>

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/medium suspension and add enough warm medium 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 half 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 a 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 medium.

Cell Maintenance

Maintain the cells as required for their growth and metabolism. Generally, one half medium 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 (100–1,000 ml).

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 and magnesium-free phosphate-buffered saline solution (CMF-PBS) or serum-free medium.
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 ~6 ml. Therefore, ~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 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 medium 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 medium.
5. The cells can be separated from the microcarriers by filtration through a suitable nylon, PTFE, or stainless steel screen.

This general protocol may be modified to reflect unique characteristics of any given cells. 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.

References

1. Varani, J., *et al*., J. Biol. Stand., **13**, 67 (1985).
2. Varani, J., *et al*., In Vitro Cell and Devel. Bio., **22**, 575 (1986).
3. Varani, J., *et al*., J. Biol. Stand., **14**, 311 (1986).
4. Varani, J., *et al*., J. Biol. Stand., **16**, 333 (1988).
5. Varani, J., *et al*., Biotechnology and Bioengineering, **33**, 1235 (1989).
6. Varani, J., *et al*., Cytotechnology, **9**, 157 (1992).
7. Hillegas, W.J., and Varani, J., Surfaced Enhanced Microcarriers for Low-Adhesion Cells. Surfaces in Biomaterials Symposium sponsored by the Surface in Biomaterials Foundation, Minneapolis, MN, October 14-16, 1992.
8. Hillegas, W.J., and Varani, J., Coatings on Microcarriers to Enhance Attachment and Growth of Anchorage-Dependent Cells. Sixth Annual Meeting of the Japanese Association for Animal Cell Technology, Nagoya University, Japan, November 9-12, 1993.
9. Varani, J., *et al*., Cytotechnology, **13**, 89 (1994).
10. Varani, J., *et al*., Small Microcarrier Aggregates Yield High Cell Density. Proceedings of ESACT/JAACT Meeting, Veldhoven, The Netherlands, September 12-16, 1994.
11. Varani, J., *et al*., J. Biomedical Materials Research, **29**, 993 (1995).
12. Varani, J., *et al*., Cytotechnology **22**, 111 (1996).

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