Product Information

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Glass microcarrier beads

Catalog Numbers **G4519, G2517, and G2892**
Store at Room Temperature

Product Description
Glass microcarrier beads provide an optimal substrate for growing many types of anchorage dependent cells in suspension. These beads are available in three densities:

1.02 g/cm^3 (Catalog Number G4519)
1.03 g/cm^3 (Catalog Number G2517)
1.04 g/cm^3 (Catalog Number G2892)

In general, lower density beads require less vigorous stirring. The 1.02 g/cm^3 density beads are particularly useful in airlift type fermenters. Glass microcarrier beads may be reused up to ten times after enzymatic or chromic acid cleaning.

Diameter: 90–150 µm

Average area: 1.1×10^{-4} cm^2/bead

Average area: 475 cm^2/g

Beads per gram: 4.3×10^6

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.

Storage/Stability
Store at room temperature.

Procedure
The following procedure can be used to prepare a 200 ml suspension in a spinner flask. Bead concentrations of 5–40 g per liter have been successfully demonstrated. Twenty grams per liter is most frequently used.

Siliconization and Sterilization
Any glass surface that may come in contact with the microcarrier cultures, such as the inside surface of the culture vessel (e.g., spinner flask) and other glassware (e.g., pipettes, bottles, etc.) should be siliconized. This prevents the microcarrier beads and cells from adhering to the glass surface. Sigmacote™ (Catalog Number SL2) is recommended for siliconization procedures using the following protocol:

1. Add a small volume of Sigmacote to clean culture vessels and other appropriate glassware.
2. Rotate the vessel or glassware to ensure that the Sigmacote covers the entire surface that may come in contact with the microcarrier beads and cells.
3. Remove excess Sigmacote from the glassware and allow to air dry.
4. Thoroughly wash the glassware in deionized tissue culture grade water. A minimum of two washings is suggested.
5. Sterilize the culture vessels and other appropriate glassware by autoclaving.
   Note: Once glassware has been siliconized, it is not necessary to treat prior to each use.

Microcarrier Bead Preparation

1. Suspend the desired amount of glass microcarrier beads in tissue culture grade water (Catalog Number W3500). Sterilize by autoclaving at 121 °C, 18 psi, for 15 minutes. Aseptic techniques should be used from this point on.
   Note: Individual autoclave performance varies. Adequate times should be validated for your equipment.
2. Discard the water and immerse the beads in a small amount of sterile cell culture medium.
3. Allow beads to soak in the medium in a 37 °C incubator for a minimum of 30 minutes. Discard the medium and resuspend the beads in fresh, warm medium. Transfer medium/bead suspension to the spinner flask.

4. Inoculate the spinner flask with the desired amount of cells and enough warm medium to make 100 ml. The attachment phase of the spinner culture is performed at a volume to facilitate cell-to-bead interactions.
   Note: The cell inoculum is generally 1×10^5 cells/ml. For a total culture volume of 200 ml, 2×10^7 cells are needed. Inoculation densities may vary with cell type.

Incubation and Stirring Speeds

1. Place the incubated spinner flask on a stir plate at 18–21 revolutions per minute for a minimum of 6 hours (12–14 hours is generally used). Adjust stirring speed as needed to prevent bead/cell slurry from forming a static layer on the bottom of the flask.
2. Add fresh medium warmed to desired incubation temperature to a final volume of 200 ml.
3. Adjust stirring speed so that the beads remain suspended in the medium.
4. Maintain the cells as required by the growth and metabolism of your cells. Generally, one half medium exchange is needed every second day.

Harvesting Cells with Trypsin

1. Allow the microcarriers to settle and decant the medium from the flask.
2. Rinse the microcarriers with a calcium and magnesium free salt solution (Catalog Number D8537 or H9394) and resuspend in a 0.25% (w/v) trypsin solution (Catalog Number T4424) prepared in calcium and magnesium free salt solution or Trypsin-EDTA solution (Catalog Number T3924). Use equal volumes of enzyme solution and cell/bead slurry.
3. Incubate in the trypsin solution for 1–5 minutes at 37 °C depending on the adhesion characteristics of the cells. The beads may require gentle trituration for maximum recovery of cells from the microcarrier surface.
4. After the cells are released from the microcarriers, the trypsin solution should be diluted with fresh serum-enriched medium to inhibit further trypsin activity.
5. Centrifuge the cells, microcarrier beads, and diluted trypsin suspension. Decant the diluted trypsin and resuspend the cells and microcarriers in fresh culture medium.
6. Cells can be separated from the microcarriers by differential sedimentation or filtration through a suitable nylon, PTFE, or stainless steel screen. Use a 75 μm mesh or a 200 sieve designation for 90–150 μm beads.

References

1. Varani, J. et al., J. Biotechnology and Bioengineering, **25**, 1359-1372 (1983).
2. Varani, J. et al., Journal of Biological Standardization, **13**, 67-76 (1985).

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