HARVESTING OF CELLS

Because CultiSpher-G is enzymatically degradable, cell harvesting is facilitated and the need to separate cells and microcarriers is eliminated.

Recommended Procedure

Collection: Collect the microcarriers and allow them to sediment for 10 minutes. Remove the supernatant.

Washing: Wash the microcarriers twice with PBS-EDTA(0.02%, w/w EDTA), 50 ml/g dry CultiSpher-G.

Digestion: Replace the PBS-EDTA with protease solution, 30 ml/g dry CultiSpher-G. Incubate at 37 °C with occasional agitation.

Harvesting: After complete dissolution of the microcarriers(30 minutes), cells are collected by centrifugation.

Important considerations

Depending on the degree of cell aggregation either Dispase or trypsin should be used. Dissolve enzyme in PBS containing Ca^{2+} and Mg^{2+}, adjust pH to 8 0.
Protease solution must to be standardized with regards to activity to achieve complete dissolution within 15 and 30 minutes.

TECHNICAL SERVICE

We will be happy to help and assist you in establishing the use of CultiSpher in your process. Please call or fax us.

CultiSpher

Macroporous Gelatin Microcarriers

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CultiSpher-G

MICROCARRIERS FOR CELL CULTURE

Microcarrier cell culture is an established technique for production of biologicals by animal cells. CultiSpher-G from Percell Biolytica has been engineered especially for large scale animal cell culture by optimizing scale-up performance.
Key features of CultiSpher-G include:

- Large surface area for cell attachment
- Minimal seeding density
- Maximum number of recoverable viable cells in each scale-up step
- Superior mechanical protection
- High thermal stability

PRODUCT DESCRIPTION

CultiSpher-G has been optimized for the culture of cells like Chinese Hamster Ovary(CHO) cells. Cell lines that have been tested for growth include CHO, Vero, MDCK, BHK 21 and L929 cells.
CultiSpher-G is designed to be used in stirred systems, for small scale spinners and in large scale tank reactors. CultiSpher-G microcarriers can be used in virtually any type of traditional culture vessel. The best results are obtained when using vessels which give even suspension of the microcarriers with gentle stirring. Suitable vessels for small scale culture include those based on rod-stirring(Techne Ltd., Cambridge, UK) or a suspended Teflon-coated bar magnet(Bellco Glass Inc,, Vineland, NJ, USA; Wheaton Scientific, Millville, NJ, USA). Equipment for large scale culture, especially designed for microcarriers, is available from most fermentor manufacturers. CultiSpher-G is based on a highly crosslinked gelatin matrix which results in excellent mechanical and thermal stability.

AVAILABILITY AND STORAGE

CultiSpher-G is supplied as a dry powder and must be hydrated and sterilized before use. CultiSpher-G is only for *in vitro* use. The following package sizes are available:

Unit Size	Catalog Number
10 g	DG-0001-OO
100 g	DG-0001-VV
500 g	DG-0001-ZZ

INSTRUCTIONS FOR USE

PREPARATION

Rehydration: The dry microcarriers are swollen and hydrated in calcium and magnesium free PBS(50 ml/g dry CultiSpher-G) for at least 1 hour at room temperature. Some of the beads might float at first, but autoclaving will expel any air trapped in the beads allowing them to settle.

Sterilization: Without removing the PBS, the microcarriers are sterilized by autoclaving (for instance 121 ^{o}C, 15 min, 15 psi).

Washing: Remove the PBS by suction, add new PBS(50 ml/g dry CultiSpher-G) and mix. Repeat the washing twice with culture medium.

Storage: Sterilized microcarriers may be stored at 4 ^{o}C for 1 month.

CULTURE PROCEDURE

Depending on the cell type and culture vessel, the exact culture procedure has to be determined for each case. CultiSpher-G is normally used at 1 g/l, incubated with 50,000-200,000 cells/ml and agitated at 15-50 RPM.
When CultiSpher-G is used at 1 g/l the yield of CHO-cells approaches $10 \cdot 10^{6}$ cells/ml. Increasing the concentration of CultiSpher-G will not result in higher cell yields unless nutrients and oxygen are supplied to an adequate level.

Recommended Procedure(100 ml culture volume)

Preparation: Prepare 0.1 g CultiSpher-G as described above.
If possible, use a medium that promotes high cell densities, for instance DMEM, with high glucose concentration. Use standard concentration of FBS, 5-10%.

Inoculation: Mix the prepared CultiSpher-G and 10^{7} cells in 50 ml prewarmed medium.

First day: Agitate gently, 30-40 RPM(Techne spinner)

Second day: Add medium to a final volume of 100 ml.

Medium exchange: Change medium frequently during this preliminary study(50 ml daily) to avoid depletion.

Important considerations

Stirring speed depends on the culture vessel and should be just sufficient to prevent sedimentation of the beads.

Cells for seeding should be harvested when they are in logarithmic growth phase.

Glass culture vessels should be siliconized prior to use.

Don't immerse the bearing of the spinner as microcarriers may circulate through it and be crushed.

Kinetics of cell attachment to gelatin is slower than to charged microcarriers, we therefore recommend a procedure with reduced starting volume, as described above.

MONITORING CELL GROWTH

Due to a large number of macropores it is not possible to examine the cells microscopically, Counting of viable cells after enzymatic digestion of the matrix is recommended. Either proteases or collagenases may be used.

Recommended Procedure

Sampling: Mix the culture and make sure that microcarriers are evenly distributed. Take duplicate samples of 0.5 ml. After sedimentation of the microcarriers, 0.3 ml of the supernatant is removed.

Dispase: Add 0.8 ml Dispase(Boehringer-Mannheim, grade II, 5 mg/ml in PBS). Mix and incubate at 37 ^{o}C until the microcarriers are dissolved(30 min).

Trypsin: if the above method results in cell aggregates, a single cell suspension may be obtained if Dispase is exchanged to trypsin(concentration has to be determined from case to case, as a start use tissue culture grade trypsin at 0.25% w/v).

Severe cell aggregation: If the cell aggregates don't disappear when trypsin is used, cell enumeration must be done by nuclei counting. After digestion of the matrix with Dispase, cells are collected by centrifugation and the supernatant discarded. A solution of crystal violet(0.01%, w/v) and Triton X-100(1%,w/v) in citric acid(0.1 M, 1 ml) is added.

Counting: As the gelatin matrix is completely dissoluble, counting can be done without interference of microcarriers. Standard techniques for cell counting(like Coulter counters and hematocytometer) are recommended.

MTT STAINING PROCEDURE

A quick procedure for direct observation of cells on CultiSpher microcarrier beads.
MTT is cleaved by an enzyme in the respiration chain in the mitochondria if live cells, generating MTT formazan a dark blue, highly visible product.

1. Extract a sample of CultiSpher from your culture vessel containing viable growing cells.
2. Load a 400 µl volume of the CultiSpher suspension to each of a desired number of wells in a 24-well plate.
3. Dissolve the MTT (3-[4,5-dimethylthiazol-2-yll-2, 5-diphenyltetrazolium bromide) in calcium and magnesium-free PBS to a final concentration of 5 mg per ml.
4. Add 40 µl of MTT to each well containing the cultured beads
5. Incubate at 37 ^{o}C for 45 minutes.
6. Observe the viable cells on the CultiSpher beads with a light or phase-contrast microscope.

Only viable cells will take up MTT and convert it to MTT formazan.
Depending on the medium in which the beads/cells are cultured, you may wish to remove the medium from the beads and replace it with a PBS rinse to enhance contrast.
MTT may be purchased from Sigma Chemical Company (Product # M-2128).