CultiSpher gelatine microcarriers

HYCLONE MEDIA AND SUPPLEMENTS

CultiSpher macroporous gelatin microcarriers provide interior surfaces for cell attachment to protect cells from shear forces. CultiSpher microcarriers are available in three varieties: CultiSpher G, CultiSpher GL, and Cultispher S. All CultiSpher microcarriers are supplied as a dry powder and must be hydrated and sterilized before use. CultiSpher microcarriers are intended for *in vitro* use only.

Key benefits of CultiSpher gelatin microcarriers include:

- Provide interior growth surface for shear-sensitive cells
- Enzymatically degradable bead simplifies cell harvest
- Transparent matrix facilitates cell count and growth monitoring

Specifications

CultiSpher G

CultiSpher G microcarriers have been optimized for the culture of cells similar in size to Chinese hamster ovary (CHO) cells. Cell lines that have been tested for growth include CHO, Vero (African green monkey kidney), MDCK (canine kidney), BHK-21 (Syrian or golden hamster kidney), and L 929 (mouse connective tissue) cells. The porosity of the CultiSpher G bead is 5–15 microns. The size of dry beads is 100–200 microns and of hydrated beads 130–380 microns.

CultiSpher GL

CultiSpher GL microcarriers are optimized for the culture of cells larger in size than CHO cells. Cell lines that have been tested for growth include 3T3 (fibroblasts), C127 (fibroblasts), and endothelial cells. The porosity of the CultiSpher GL bead is 15–25 microns. The size of dry beads is 100–200 microns and of hydrated beads 130–380 microns.

Cultispher S

Cultispher microcarriers can be used for the culture of cell lines that differ widely in their size. Cell lines that have been tested for growth include CHO, C127, and Vero cells. Due to enhanced cross-linking, a higher thermal stability and faster cell attachment have been obtained. The porosity of the CultiSpher S bead is 10-20 microns. The size of dry beads is 100–200 microns and of hydrated beads 130–380 microns.

Instructions for use

A. Preparation

- Rehydration: the dry microcarriers are swollen and hydrated in phosphate buffered saline (PBS) without calcium or magnesium (50 mL/g dry CultiSpher) for at least 1 h at room temperature
- Sterilization: sterilize by autoclaving (at 121°C, 15 min.) in the PBS used in the rehydration step
- Wash: decant the PBS, add fresh, sterile PBS (50 mL/g dry CultiSpher) and mix. Repeat the wash step twice using culture medium instead of PBS
- Storage: sterilized microcarriers can be stored at 4°C for 1 month

B. Culture procedure

The exact culture procedure should be determined for each case depending on the cell type and culture vessel. CultiSpher is normally used at 1 g/L, incubated with 50 000–200 000 cells/mL and agitated at 15–50 RPM. When CultiSpher is used at 1 g/L, the yield of CHO-cells approaches 10^7 cells/mL. Increasing CultiSpher concentration will not result in higher yield unless nutrients and oxygen are increased to adequate levels.

Recommended procedure (100 mL culture volume):

- Preparation: prepare 0.1 g CultiSpher as described above
- Medium: use a culture medium that promotes high cell density, for example, DME with high glucose concentration. Use a standard concentration of fetal bovine serum (FBS) 5%–10%
- Inoculation: mix CultiSpher and 10^7 cells in 50 mL prewarmed medium (for CultiSpher S, use 2×10^7 cells)
- First day: agitate gently (e.g., 30–40 RPM with a stirrer)
- Second day: add medium to a final volume of 100 mL (with CultiSpher S, increase stirring speed to 50 RPM)
- Medium exchange: change medium frequently during this preliminary study (50 mL daily) to avoid nutrient depletion

Important considerations:

- Stirring speed depends on the culture vessel and should be just sufficient to prevent sedimentation of the beads. Increased stirring speed is recommended in later stages of the culture to facilitate oxygen transfer
- Cells should be harvested in their logarithmic growth phase
- Glass culture vessels must be siliconized prior to use
- Do not immerse the bearing of the stirrer, as microcarriers might circulate through it and be crushed
- Because the kinetics of cell attachment to gelatin is slower than to charged microcarriers, we recommend using the reduced volume as described above

C. Monitoring cell growth

Due to the large number of macropores, it is difficult to examine the cells microscopically. Counting of viable cells after protease or collagenase digestion of the matrix is recommended. Cells can be observed on the beads by using MTT staining (see MTT staining procedure).

Recommended procedure:

- Sampling: mix the culture and make sure that microcarriers are evenly distributed. Take duplicate samples of 0.5 mL. After sedimentation, remove 0.3 mL of the supernatant
- Digestion, standard: add 0.8 mL Dispase™ II 5 mg/mL in PBS (Roche). Mix and incubate at 37°C until the microcarriers are dissolved (approximately 15–45 min)
- Cell aggregates: if the digestion method results in cell aggregates, a single cell suspension can be obtained by using trypsin instead of Dispase. The concentration must be determined for each case. Initially, use tissue culture grade trypsin at 0.25% w/v
- Severe cell aggregation: if cell aggregates do not disperse with trypsin, enumerate nuclei by direct staining. After digestion of the matrix with Dispase, collect cells by centrifugation and add crystal violet (0.01% w/v) and Tween™ 20 (1% w/v) in citric acid (0.1 M, 1 mL) to stain nuclei
- Counting: the gelatin matrix is completely soluble and counting can be performed without interference. Standard techniques for cell counting including flow cytometer or hematocytometer are recommended. See MTT staining procedure for a technique to observe cells on the beads

D. Harvesting of cells

Because CultiSpher microcarriers are enzymatically degradable, cell harvesting is facilitated with no need to separate the cells from microcarriers.

Recommended procedure:

- Collection: allow microcarriers to sediment for 10 min. Remove the supernatant
- Wash: wash microcarriers twice with PBS-EDTA (0.02%, w/w EDTA) at 50 mL/g dry weight CultiSpher
- Digestion: replace the PBS-EDTA with protease solution, 30 mL/g dry weight CultiSpher. Incubate at 37°C with occasional agitation
- Harvesting: after dissolution of the microcarriers (15–45 min), collect cells by centrifugation

Important considerations:

- Depending on the degree of cell aggregation, either Dispase or trypsin can be used. Dissolve enzyme in PBS containing Ca^{2+} and Mg^{2+}, adjust pH to 8.0
- Protease solution must be standardized to consistently dissolve the microcarriers within 15 to 45 min

MTT staining procedure

A quick procedure for direct observation of cells on CultiSpher microcarriers using 3-[4,5-dimethylthiazol-2-y1]-2, 5- diphenyl-tetrazolium bromide (MTT).

For cell observation, use this simple procedure:

1. Extract a sample of CultiSpher microcarriers with attached cells.
2. Dispense 400 µL to each of a desired number of wells in a 24-well plate.
3. Dissolve MTT in calcium-and magnesium-free PBS to a final concentration of 5 mg/mL.
4. Add 40 µL of MTT to each well.
5. Incubate at 37°C for 45 min.
6. Observe the viable cells on the microcarriers using any standard light or phase-contrast microscope.

Technical notes:

- Viable cells take up the MTT compound and convert it into a dark blue, highly visible product
- Depending on the culture medium, you might wish to remove it from the beads and replace it with a PBS rinse to enhance contrast

Ordering information

Product	Size	Product code
CultiSpher G	100 g	SV30008.03
	500 g	SV30008.04
CultiSpher GL	100 g	SV30006.03
	500 g	SV30006.04
CultiSpher S	100 g	SV30009.03
	500 g	SV30009.04

Related products	Size	Product code
PBS	100 mL	SH30028.01
	500 mL	SH30028.02
	1000 mL	SH30028.03
DME, high glucose	500 mL	SH30022.01
	1000 mL	SH30022.02
FBS	100 mL	SH30070.02
	500 mL	SH30070.03
	1000 mL	SH30070.04
Trypsin	100 mL	SH30042.01
	500 mL	SH30042.02

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