

# Cytopore

## Instructions for Use

Microcarrier culture is a technique which makes possible the high yield culture of anchored dependent cells. Cytopore™ microcarriers are macroporous, they supply an even larger surface area per volume to enhance this already high yielding culture technique. The macroporous structure allows growth of the cells inside the microcarriers, protecting them from shear forces.

### Applications

Cytopore microcarriers are designed for use in stirred suspension culture systems for the growth of adherent recombinant CHO cells and the production of recombinant proteins for therapeutic use. Cytopore 1 has a charge density of 1.1 meq/g, while Cytopore 2 has been optimized for anchorage-dependent cells requiring a charge density around 1.8 meq/g. This development does not exclude any of the applications that have been tested on Cytodex® microcarriers, or any future applications.

Cytopore is intended for the final production stages of cell culture. The difficulty of removing the cells from the porous structure makes them less suited to scale up procedures where cells are cultured and harvested and then used as inoculum for the following culture step.

### Properties

Cytopore is designed to meet the requirements for adhering CHO cells to microcarriers. It has a macroporous structure with a microporous matrix. The matrix is based on a cross-linked cellulose which is substituted with positively charged N,N,-diethylaminoethyl groups. The charged groups are distributed throughout the microcarrier matrix.

The microporous matrix supplies nutrients to the cells from both the apical and basolateral sides. A result of the macroporosity is the high ratio of surface area to volume which allows very high cell densities. It gives the cells easy access to the interior of the carrier upon inoculation. This provides the cells with protection from shear forces, enabling an increase in aeration and stirrer speed, which in turn permits the use of higher microcarrier concentrations, thereby achieving higher cell densities. It is easy to maintain the microcarrier in suspension as only gentle stirring is required, however, the microcarriers are easily separated from the medium.

Cytopore particle size deviation is very small and therefore provides for even suspensions and simultaneous reaching of confluence. The cellulose matrix is non-toxic to the cells and is biodegradable after use. In swollen conditions the microcarriers are tough, they keep their shape and can tolerate mechanical stress. They are also autoclavable.

### Specifications of Cytopore

Particle diameter d50 (µm)	200-280 µm <sup>1</sup>
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Effective surface area	1.1 m <sup>2</sup> /g dry <sup>1</sup>
Relative density <sup>2</sup>	1.03 g/mL <sup>1</sup>
Average diameter of pore openings	30 µm <sup>1</sup>
Volume	40 mL/g dry

<sup>1</sup> Data from Ashai Chemical Industry Co. Ltd.

<sup>2</sup> In 0.9% NaCl

### Instruction for use

Before proceeding to stirred cultures, we recommend that initial experiments and evaluation studies be performed in stationary cultures.

### Preparation

Hydrate and swell the dry microcarriers in PBS (50–100 mL/g of Cytopore) in a siliconized bottle. Some of the carriers might float at first, but autoclaving will expel any air trapped in the carriers allowing them to settle. Gently agitate the solution occasionally, for approximately 10 minutes at room temperature. Adjust pH to 7–7.5 and then autoclave the microcarriers at 121 °C for 20 minutes.

After autoclaving allow the sterilized microcarriers to settle, remove the supernatant and add fresh PBS. A second wash with PBS is recommended.

Cytopore is very stable and even three autoclavings do not affect cell culture performance. The carriers can be sanitized with 70% ethanol, they also withstand 1% NaOH solutions.

Prior to use, allow the sterilized microcarriers to settle, decant the supernatant and rinse the microcarriers with a serum containing culture medium. This will coat the microcarriers with attachment proteins. Occasionally agitate the microcarrier solution for 10 minutes. Allow the microcarriers to settle before adding new culture medium. Suspend the microcarriers uniformly and, preferably, leave them overnight in a medium containing serum. Resuspend the microcarriers and then transfer the required volume of homogeneous slurry to the culture vessel.

### Culture vessels

Full details of culture vessels can be found in “Microcarrier Cell Culture: Principles and Methods”.

Generally, microcarrier cultures can be contained in virtually any type of culture vessel. However, best results are obtained with equipment that gives even suspension of the microcarriers with gentle stirring. The most suitable vessels for general purpose microcarrier culture are those with efficient gassing and mixing systems that do not generate high shear forces and provide a homogeneous culture environment.

For really high cell densities a perfusion culture system is needed. However, when selecting a vessel for a perfusion culture some design criteria need consideration. The stirrer should never come into contact with the inside surface of the vessel during culture because it may damage the microcarriers. Similarly, spinner vessels with a bearing immersed in the culture medium are not suitable because the microcarriers can circulate through the bearing and get crushed. Alternatives to fermentors for perfusion culture exist for laboratory, pilot and production scale applications.

**Note:** Glass culture vessels should be siliconized before use.

## Culture procedure

The exact culture procedure depends on the type of cell and on the culture vessel. Macroporous microcarrier cultures normally contain 1–2 g Cytopore/L, and are usually inoculated with about  $2 \times 10^5$  cells/mL. Perfused cultures may contain much higher microcarrier concentrations. In such instances the inoculum should be increased proportionally.

## Agitation during initial culture stage

Successful microcarrier culture depends on the state of the inoculum and correct operation during the initial stages. Conditions vary with cell type and the culture conditions. Anchorage-dependent cells cannot survive unattached in suspension for very long.

The easy access to the interior of the carriers facilitates initiation of the culture at full culture volume, and enables continuous stirring at 30 rpm, from commencement of the culture.

## Microcarrier density

- For a stationary culture, cover the bottom of a bacteriological petri dish with microcarriers. The suggested starting concentration of microcarriers for a 60 mm  $\emptyset$  petri dish is approximately 2 mg/mL (about 0.1 cc/mL).
- For stirred cultures, the optimum concentration varies from cell to cell. The concentration for CHO cells is approximately 1–2 mg/mL. However, this very much depends on the feeding strategy for the culture.

## Culture maintenance

The high cell density experienced with macroporous carriers means that the culture rapidly consumes any available metabolites. A steady state should be maintained; toxic metabolites should not be allowed to accumulate and pH values should be maintained at the set level. Rapid changes in pH cause cell peeling and a reduction in the final cell yield.  $dO_2$  should also be kept at the desired level.

## Monitoring cell growth

Cell growth can be monitored by glucose consumption and lactate build-up,  $dO_2$  consumption, and cell counting.

## Cell counting

Step	Action
1	Place 1 mL of suspended microcarriers in a centrifuge tube used for sedimentation.

Step	Action
2	When the carriers have settled, remove the supernatant and add PBS.
3	Allow the carriers to settle again, then remove the PBS.
4	Add 0.1 M citric acid, 0.1% crystal violet, to give a final volume of 1 mL. Incubate at 37 °C for 2–4 hours. To generate shear force to remove the cells from the carriers, use an injection needle to make a homogenous cell/microcarrier suspension.
5	After incubation, count the number of nuclei in a haemocytometer.

## Observation of cell adhesion

a) By using an optical microscope.

Step	Action
1	Place a 1 mL sample of a well suspended culture in a 35 mm $\emptyset$ petri dish.
2	Add 0.1 mL of MTT <sup>1</sup> solution.
3	Incubate for 4 hours at 37 °C in an incubator.
4	The reactive, live cells are dyed black and can be monitored using a microscope.

Cells can also be stained with haematoxylin and cell attachment to the microcarriers can then be monitored with a light microscope.

## Principle of function

MTT is cleaved by an enzyme in the respiration chain in the mitochondria of live cells, generating MTT formazan (black needle shaped crystals).

It is also possible to observe the cells using a confocal microscope. T. Mori, K. Konomoto, J. Shirokaze, K. Sagara, *New method to observe cells inside macroporous microcarriers*. Ashai Chemical Industry Co., Ltd.

b) By using SEM (Scanning Electron Microscopy).

Step	Action
1	Place 2 mL of microcarrier suspension in a centrifuge tube.
2	Allow the carriers to settle then remove the supernatant.
3	Wash once with PBS.
4	Remove the supernatant.
5	Add 2% glutaraldehyde/PBS solution and incubate overnight at 4°C.
6	Clean twice with PBS, dehydrate with alcohol, then substitute with isoamyl acetate and perform critical point drying with liquified carbon dioxide.
7	Observe cell adhesion to the microcarriers.

**Note:**  
Cleaning and hydration must be performed very gently otherwise cells detach from the microcarriers.

c) Observation of the cells inside the microcarrier. This is performed by cutting sections of the carriers with a microtome.

<sup>1</sup> MTT solution: dissolve MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in PBS free of  $Ca^{2+}$  and  $Mg^{2+}$ , at a concentration of 10 mg/mL

## Materials

PBS  
2% glutaraldehyde/PBS  
Resin (Quetol 523M, Nisshin EM)  
Gelatine capsule

## Procedure

Step	Action
1	Place the required quantity of microcarriers in a centrifuge tube, allow them to settle and remove the supernatant.
2	Clean twice with PBS.
3	Remove the supernatant and add an appropriate amount of 2% glutaraldehyde/PBS.
4	Leave for 6 hours or more so that the cells are properly chemically fixed to the microcarriers.
5	Clean twice with PBS.
6	Resin substitution. Adjust to 50, 70, 90, 95 and 100% resin in PBS. Substitute twice for each concentration. Keep shaking and leave for 1 hour.
7	Substitute with mixed resin solution (mixture of Quetol 523 M 100 g and QCU-1 0.05 g) shake occasionally and leave for 2 hours. Repeat this once.
8	Take the mixed resin solution and place it in a gelatine capsule.
9	Leave overnight in a cool and dark place.
10	Incubate at 60 °C for 12 hours to harden.
11	Dry cut the resin with the embedded microcarriers on a super microtome.
12	Stain the solution with Giemsa dye and observe under a microscope.

## Harvesting cells

There is very limited data on subcultivation steps. The data available indicate that it may be possible to harvest cells with 0.25% trypsin, releasing most of them from the microcarriers.

## Quality control

Each batch of Cytopore is subjected to stringent quality control tests to examine both physicochemical and functional properties. Each lot is function tested with CHO-K1 cells in a stirred culture. A certificate of analysis stating the results of these tests is available on request for all batches of Cytopore.

## Availability and storage

Cytopore is supplied as a dry powder and must be hydrated and sterilized before use. Unopened packs of Cytopore stored in dry conditions are stable for more than eight years. Cytopore which has been hydrated and sterilized as described above, can be stored sterile in PBS for at least five years.

## Ordering Information

Product	Pack size	Ordering number
Cytopore 1	20 g	17-0911-01
Cytopore 1	100 g	17-0911-02
Cytopore 1	500 g	17-0911-03
Cytopore 2	20 g	17-1270-01
Cytopore 2	100 g	17-1270-02
Cytopore 2	500 g	17-1270-03
Cytopore 2	1000 g	17-1270-04

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