Cytoline 1 and Cytoline 2 Macroporous microcarriers

Cytoline™ microcarriers are designed for use in packed, fluidized bed reactors for the culture of CHO and suspension cells intended for the production of recombinant proteins for therapeutic use. Cytoline microcarriers are macroporous and have a matrix based on polyethylene which is weighted with silica. The microcarriers yield high cell concentrations which can be sustained over long periods of time.

- Macroporous microcarriers
- High density cell culture
- Designed for fluidized bed cultures

Macroporous microcarriers

Cells gain easy access to the interior of Cytoline due to the macroporous structure of the microcarriers (Figure 1). Inside Cytoline, cells are protected from both physical and environmental stresses, and are able to create their own micro-environment. This micro-environment increases metabolic communication between the cells and permits protein-free perfusion, which in turn decreases media costs, particularly in long-term continuous processes.

The polyethylene base matrix causes the microcarriers to be hydrophobic while the silica content gives Cytoline a slight negative charge. Cytoline is further primed by an NaOH wash during preparation to optimize performance, the effects of which are particularly noticeable in protein-free media.

High density culture

Cytoline microcarriers offer both an external surface and an interior space which can be populated by anchorage-dependent cells or cells grown in suspension. Cell retention during perfusion is improved due to metabolic communication. As a result, cell density per reactor volume is high in contrast to when conventional microcarriers are used (Figure 2).

Fig. 1. Scanning electron micrograph of Cytoline 1.

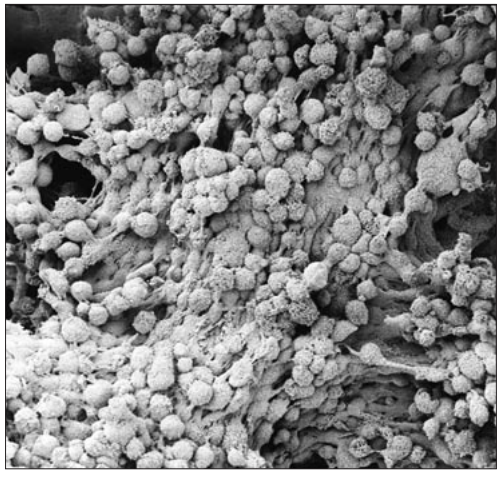

Fig. 2. Close-up reveals the tight clustering of CHO cells on Cytoline 1.

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Designed for fluidized bed culture

Cytoline 1 is optimized for the culture of CHO cells in fluidized beds. CHO cells attach well to Cytoline 1 and final cell densities are very high. These high densities are possible because of the high sedimentation rate of Cytoline 1, 120 to 220 cm/min. A high sedimentation rate enables the use of a high circulation rate which ensures an adequate supply of oxygen to the reactor in order to maintain the high cell concentration.

Cytoline 2 is primarily intended for the culture of hybridomas (Figure 3) and other stress-sensitive cells in packed fluidized beds. (Cytoline 2 supports a lower density of cells than Cytoline 1.) The sedimentation rate for Cytoline 2 is 25 to 75 cm/min, which means that the circulation rate is lower and shear stress on the cells is less. The lower sedimentation rate also allows Cytoline 2 to be used in stirred cultures.

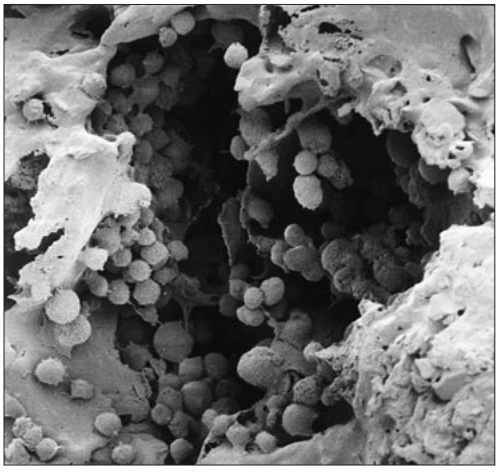

Fig. 3. Human hybridoma cells cultured within Cytoline 2.

Good adhesion during long periods of culture

When culturing recombinant CHO cells using Cytodex™ microcarriers, the cells occasionally have a tendency to peel off the microcarriers after about 10 days in culture. When using Cytoline to culture CHO cells, there is no sign of cell detachment even after more than 60 days in culture.

Characteristics of Cytoline microcarriers

- Steam sterilizable (in situ) 121 °C, 1 bar
- Alkali and acid resistant
- Lot-to-lot consistency
- No material of biological origin is used
- Suitable for immobilization and growth of adherent and non-adherent cell types
- Suitable for bioreactor systems that are in use in industrial environments, such as stirred tanks, airlift culture systems and fluidized beds
- Increases culture surface area when used in routine laboratory culture techniques, such as roller, spinner culture or shaker flasks
- Lens-shape advantageous for nutrient /oxygen supply diffusion
- Storage in the dark gives optimal shelf-life

Specifications of Cytoline

	Cytoline 1	Cytoline 2
Sedimentation velocity (cm/min)	120–220	25–75
Length (mm)	1.7–2.5	1.7–2.5
Thickness (mm)	0.4–1.1	0.4–1.1
Density (g/cm^3)	1.32	1.03
Pore size (µm)	10–400	10–400
Surface area (m^2/g)	>0.3	>0.1

Preparing Cytoline

– General washing procedure

1 To the desired volume of Cytoline (1 or 2), add twice the amount of distilled water and autoclave the mixture for 10 min at 121 °C (1 bar). The maximum temperature the microcarriers can withstand is 121 °C.

2 Sieve the mixture to remove the water supernatant, then add twice the amount of fresh distilled water.

3 Stir the microcarrier/water suspension for 10 min.

4 Repeat 3 times steps 2 and 3.

5 Add an equal volume of 0.1 M NaOH to the microcarriers and incubate this mixture overnight.

6 Wash 3 or 4 times with distilled water until all alkali is removed (check the pH of the wash water).

7 Autoclave the microcarriers in distilled water or PBS at 121 °C (1 bar) for 30 min.

– Testing the microcarrier

1 Transfer 2 ml of dry Cytoline to either 125 ml spinner flask or 100 ml shaker flask.

2 Add 10 ml of distilled water and autoclave the mixture for 10 min at 121 °C.

3 Sieve the mixture to remove the water supernatant, then add 10 ml of distilled water.

4 Stir the microcarrier/water suspension for 10 min.

5 Repeat this procedure 3 times.

6 Add 10 ml of 0.1 M NaOH to the microcarriers and incubate this mixture overnight.

7 Remove alkali using the same wash procedure as outlined above.

8 The final wash should have a pH below 8.5 or close to that of the added distilled water.

9 The microcarriers are now ready to be autoclaved.

NOTE. Do not use temperatures higher than 121 °C (1 bar). Never autoclave the carriers without any liquid.

Washing of the microcarriers inside the fluidized bed reactor is described in the User Manual for Cytopilot™ Mini (Code No. 18-1114-41).

Ordering information

Designation	Pack size	Code No.
Cytoline 1	50 ml	17-1268-01
Cytoline 1	500 ml	17-1268-02
Cytoline 1	5 litres	17-1268-03
Cytoline 2	50 ml	17-1269-01
Cytoline 2	500 ml	17-1269-02
Cytoline 2	5 litres	17-1269-03

to order:

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