Cytodex™

MICROCARRIERS

Animal cell culture is vital to the study of cell structure, function, and differentiation. Additionally, it provides important biologicals for the pharmaceutical industry, including viral vaccines, enzymes, hormones, and antibodies. In microcarrier cell culture technology, anchorage-dependent animal cells are grown on the surface of small (~ 150 µm diameter) spheres that are maintained in stirred suspension cultures. Due to their extremely high surface area to volume ratio, microcarriers are an attractive alternative to conventional monolayer cell culture methods, such as roller bottles or cell factory culture systems. Microcarrier technology has been used successfully for:

- Routine and high density cell culture
- Research applications
- Production culture volumes > 1000 L
- Efficient monitoring and culture control
- Reduction of costs and contamination in cell culture applications

Applications

The use of Cytodex™ microcarriers enables most anchorage-dependent animal cells to grow in suspension cultures, in either a batch or perfusion culture format. Additionally, they can be used to increase the surface area of traditional monolayer cultures.

In stirred suspension cultures, cells grow in a homogeneous environment where the culture parameters are easily monitored and controlled. Cultures can be sampled periodically to examine cell morphology and to determine cell viability. Microcarrier techniques are therefore a logical choice in applications where anchorage-dependent cells are used for the production of a range of biological therapies.

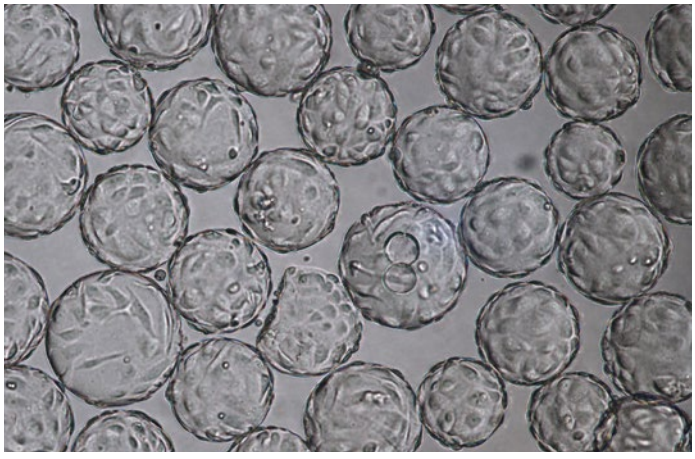


Fig 1. Vero cells growing on Cytodex 1.

Cytodex microcarriers have been used in a variety of research applications including:

- Studies of cell structure, function, and differentiation
- Enzyme-free subcultivation
- Implantation studies
- Harvesting of mitotic cells
- Light and electron microscopy
- Transportation and storage

Industrial-scale applications utilizing Cytodex include the production of cells, cell products, and viral vaccines (see industrial applications).

Properties and characteristics

Two types of standard Cytodex are available to support the growth of anchorage-dependent animal cells for use in a range of applications. Both trypsin of microcarriers are designed to meet the special requirements for this technology.

Cytodex bead size and density are optimized to support maximum cell growth rate and cell yield. The biologically inert matrix provides a stable, but non-rigid substrate for stirred cultures from which cells are easily harvested. Cytodex is transparent, allowing microscopic examination of attached cells.

The physical characteristics of Cytodex are shown in Table 1.

Table 1. Characteristics of Cytodex

	Cytodex 1	Cytodex 3
Density[1] (g/mL)	1.03	1.04
Particle size, d_{50v}[1,2,3] (µm)	~ 180	~ 175
Particle size distribution[1,2,4] (µm)	131 to 220	133 to 215
Approximate area (hydrated)[1,2] (cm^2/g dry weight)	4400 cm^2/g	2700 cm^2/g
Approximate number of microcarriers/g dry weight[2]	6.8×10^6	4×10^6
Hydration factor[1,2] (mL/g dry weight)	18	14
Sedimentation velocity[2,5]	90 cm/h	120 cm/h

[1] In 0.15 M NaCl solution
[2] Some batch-to-batch variations may exist
[3] Median particle size of the cumulative volume distribution
[4] ≥ 70% volume share within given range
[5] Measured in a 64 × 370 mm measuring cylinder using 3 g/L in 0.15 M NaCl solution. Values measured on empty microcarriers without cells

Cytodex 1, formed by substituting a cross-linked dextran matrix with positively charged DEAE* groups distributed throughout the matrix, is a general purpose microcarrier with an ionic capacity of 1.4 to 1.6 mmol Cl^-/g dry substance. It is particularly suitable for established cell lines and for production of viruses or cell products from cultures of primary cells and normal diploid cell strains.

* N, N-diethylaminoethyl

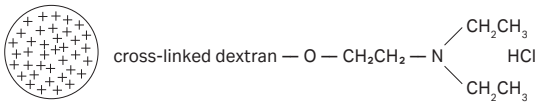
Cytodex 3, formed by chemically coupling a thin layer of denatured collagen to the cross-linked dextran matrix, is the microcarrier of choice for cells that may be difficult to culture in vitro, and particularly for cells with an epithelial-like morphology. Because the collagen surface layer can be digested by a variety of proteolytic enzymes, it provides novel opportunities for harvesting cells from the microcarriers while maintaining maximum cell viability and membrane integrity. These issues may be critical in developing successful serial subcultivation protocols required for scaling-up culture volumes.

Figure 2 is a schematic representation of the two types of Cytodex. Cytiva produces Cytodex in accordance with ISO 9001, and every batch must conform to stringent specifications. Quality control tests are performed to satisfy both physiochemical and functional (i.e., cell growth) properties. A certificate of analysis (COA) is available on request.

Both Cytodex 1 and Cytodex 3 are also available gamma irradiated and pre-sterilized under the names Cytodex 1 Gamma and Cytodex 3 Gamma.

See separate instructions for use *Cytodex 1 and Cytodex 3 Gamma microcarriers – Instructions for use.*

Cytodex 1. Charges throughout the matrix.



Cytodex 3. Collagen layer coupled to surface.

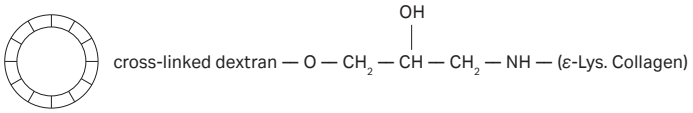


Fig 2. Schematic representation of the two types of Cytodex.

Culture procedure

The exact culture requirements (e.g., medium, serum, and supplements) depend on the cell type, while the size of the final culture volume and design of the culture vessel determine the optimal culture procedure. Culture parameters that should be optimized are listed in the *"Principle and methods -Microcarrier cell culture Handbook"* – Development of a microcarrier process and Troubleshooting sections" available from Cytiva.

Cytodex, supplied as a dry powder, must be hydrated and sterilized before use. Autoclaving is a straightforward method for sterilization.

Batch-type microcarrier cultures usually contain up to 5 g of Cytodex per liter of culture volume. Fed-batch and perfusion cultures can sustain higher cell densities, and Cytodex concentrations may be greater than 20 g/L.

Monitoring cell growth

Representative samples of the microcarrier culture can be withdrawn at any time during the culture period to monitor cell growth and morphology. The cells on the microcarriers can then be visualized directly by phase contrast microscopy or by light microscopy after staining with a suitable dye such as hematoxylin.

One widely used method for determining cell number is the nucleus extrusion method. In this procedure, cells on microcarriers are lysed using a hypotonic solution and then the nuclei counted. Counting can either be performed manually, using a hemocytometer, or automated using, for example, an NC200 from Chemometec.

The recommended method for determining cell number is the nucleus extrusion method. In this procedure, cells on microcarriers are lysed in 0.1 M citric acid, and the released nuclei are stained with crystal violet and then counted in a hemocytometer (1).

Alternatively, cells can be harvested (see below) and then counted by microscopy. A third method for cell enumeration is to determine and relate nucleic acid concentration to cell number using quantitative assays.

Harvesting cells

The common methods for harvesting cells from Cytodex involve standard procedures employing proteolytic enzymes, such as our recombinant trypsin, HyrTrp™ trypsin. Pre-washing confluent microcarriers with a solution of EDTA-PBS* prior to trypsinization improves the harvested yield of strongly adherent cells. The harvesting procedure should be optimized and standardized with respect to the activity of the enzyme solution, the temperature, and the duration of exposure. Other procedures include the use of chelating agents, exposure to hypotonic conditions, cold conditions, sonication, and alteration of the surface tension of the culture medium.

* Ethylenediamine tetraacetic acid – phosphate buffered saline

Scaling up by serial subcultivation

Serial subcultivation of cells on microcarriers is a cost-effective means for establishing animal cell culture production facilities. It is achieved using a series of culture vessels of increasing volume and capacity.

In this procedure, cells cultured in one vessel are subsequently used to inoculate the following, larger vessel in the series. This approach to scale-up eliminates large numbers of culture vessels, such as roller bottles or multi-trays, that would otherwise be required to obtain sufficient cells for inoculating the larger production-scale bioreactors.

The protocol for serial subcultivation of cells from one culture of Cytodex to the next should established optimized at small-scale prior to scaling up to production-culture volumes. Because some cell types can undergo bead-to-bead migration with Cytodex, subcultivation can be performed without a harvesting step using proteolytic enzymes. In this case, scale-up can be achieved simply by increasing the culture volume and adding more Cytodex.

Cells incapable of bead-to-bead migration, however, must first be harvested from the confluent/semi confluent culture, as outlined above, for inoculation during scale upRoutine procedures for harvesting these cells as a single cell suspension – as opposed to clumps of cells – with maximum viability, retained membrane integrity, and high attachment efficiency, will greatly facilitate the successful inoculation of the following culture and thereby the entire scale-up process.

Equipment recommendations

Microcarrier cultures can be contained in virtually any type of cell culture vessel that can be kept sterile. However, for maximum cell yield and productivity from general purpose microcarrier cultures, it is recommended to use equipment with efficient mixing systems that provide a homogeneous culture environment, a uniform suspension of microcarriers, and an adequate supply of oxygen. Additionally, high shear forces should be avoided due to the shear stress-sensitivity of animal cells.

For routine, small-scale microcarrier cultures (up to a few liters working volume), some commercial suppliers have modified traditional glass spinner vessels containing a magnetically driven stirring rod or impeller, specifically for use with microcarriers.

Note: Glassware with which Cytodex comes into contact should be siliconized before use.

Laboratory-scale bioreactors designed for animal cell cultures are now widely available from various commercial suppliers. These bioreactorsfitted with probes and control systems for monitoring pH, temperature, and dO_2, permit essential culture parameters to be optimized at small-scale, for future reference at production-scale. Also, the availability of perfusion systems allows for simplified, long-term productivity studies.

Further information and recommendations on the design and optimization of production-scale culture processes that incorporate microcarriers are available from Cytiva.

Industrial applications

Industrial-scale Cytodex based cell culture has proven to be a reliable and cost-effective process for the manufacture of both human and animal health care products.

Significant savings include a greater than 50% reduction in culture medium and serum costs, reduced labor costs, and decreased risks of contamination.

System at a working volume of 100 L containing 3 g of Cytodex per liter can be handled by one operator and provides a surface area for cell growth equivalent to that of a facility containing 1650 × 800 cm^2 roller bottles or 220 × 6000 cm^2 multi-tray units. A single-use bioreactor suunit culture fermentation.

Because of rapid developments in recombinant DNA technology for the expression of foreign proteins in animal cells, the use of transformed mammalian cells, such as CHO, CI27, Vero, COS, CV-1, etc., is increasing to compete with bacterial and yeast expression systems. With microcarrier cell culture technology, sufficiently large numbers of anchorage-dependent animal cells can be cultured at production-scale to meet the needs of the pharmaceutical industry.

Virus vaccines, interferons, plasminogen activators, urokinases, hormones such as animal and human growth hormones, and a variety of factors such as platelet-derived growth factor (PDGF), epithelial growth factor (EGF), tumor necrosis factor (TNF), erythropoietin (EPO), colony stimulating factor (CSF), and others are produced from such microcarrier facilities.

Some of these products are available on the market as human and animal diagnostics and therapeutics. Many other products are currently undergoing evaluation in clinical trials, and even more are in the developmental process.

Reference:

1. Sanford, K. K. *et al.*, The Measurement of Proliferation in Tissue Cultures by Enumeration of Cell Nuclei. *J. Nat. Cancer Inst.* **11**, 773–795 (1951).

Ordering information

Product	Quantity	Product code
Cytodex 1 gamma (dry powder)	30 g	17548701
Cytodex 1 gamma (dry powder)	300 g	17548702
Cytodex 1 gamma (dry powder)	3 kg	17548703
Cytodex 3 gamma (dry powder)	30 g	17548801
Cytodex 3 gamma (dry powder)	300 g	17548802
Cytodex 3 gamma (dry powder)	3 kg	17548803
Cytodex 1 (dry powder)	25 g	17044801
Cytodex 1 (dry powder)	100 g	17044802
Cytodex 1 (dry powder)	500 g	17044803
Cytodex 1 (dry powder)	2.5 kg	17044825
Cytodex 1 (dry powder)	5 kg	17044804
Cytodex 3 (dry powder)	10 g	17048501
Cytodex3 (dry powder)	100 g	17048502
Cytodex 3 (dry powder)	500 g	17048503
Cytodex 3 (dry powder)	2.5 kg	17048525
Cytodex 3 (dry powder)	5 kg	17048504

Packs of Cytodex stored unopened under dry conditions are stable for eight years. Cytodex that has been hydrated and sterilized can be stored sterile in phosphate buffered saline (PBS) for at least two years at 4°C.

cytiva.com/bioprocess

For local office contact information, visit cytiva.com/contact

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